

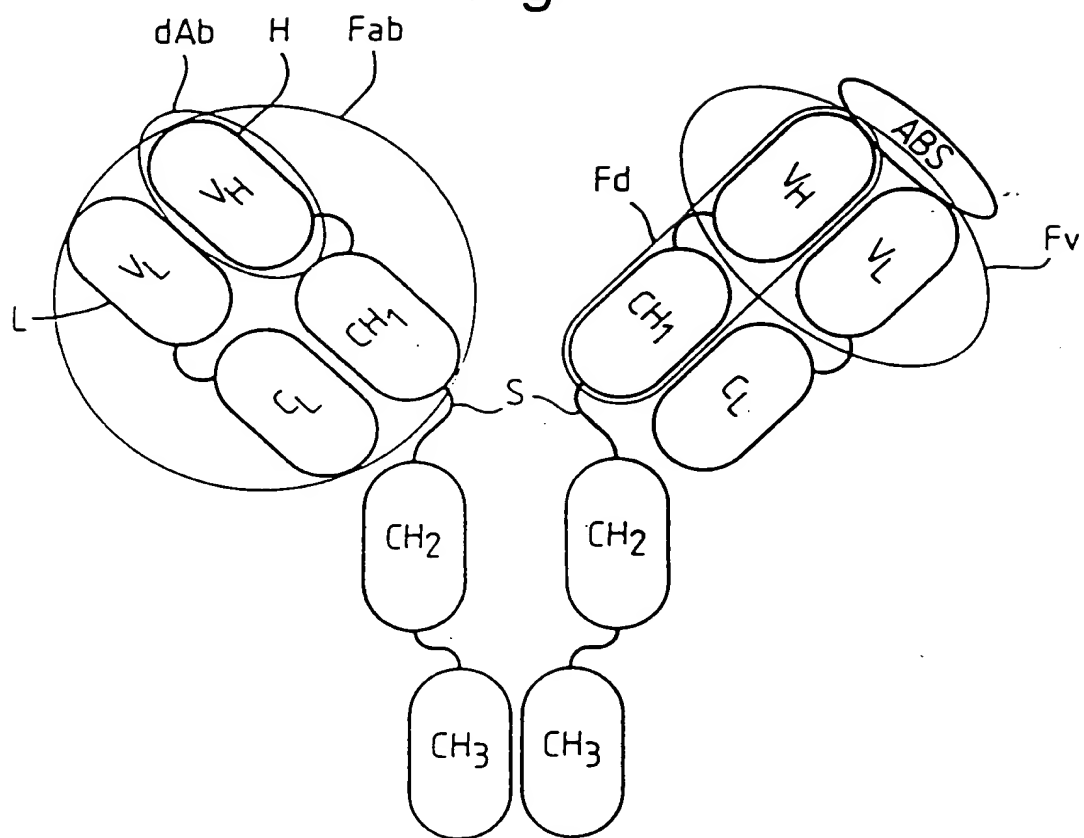
[illegible]

Fig.2 (i).

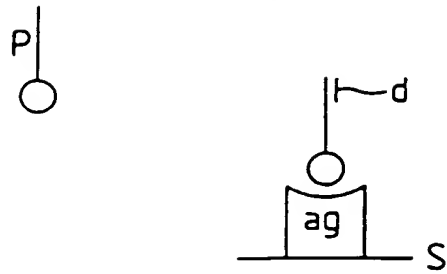


Fig.2 (ii).

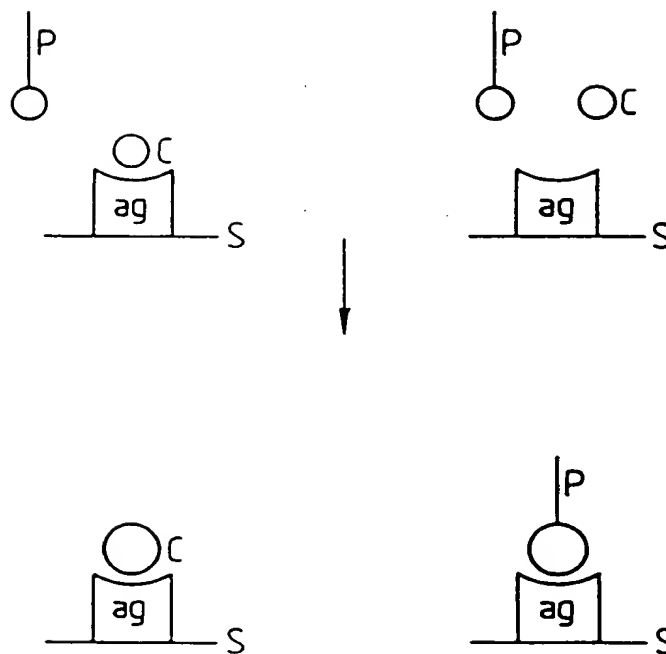
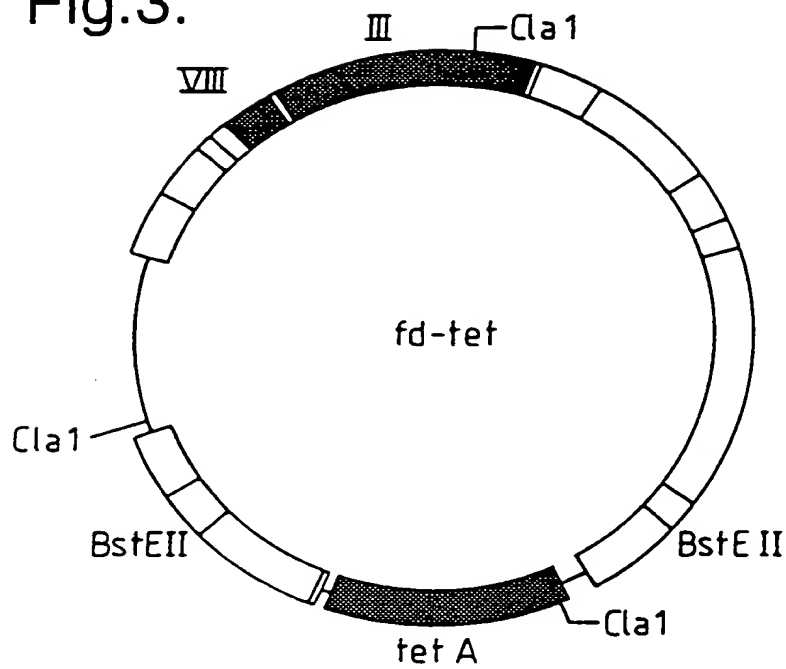


Fig.3.



fd - tet

~

cleave with BstEII

~

fill in with Klenow

~

re-ligate

↓

FDT 6 Bst

~

in vitro mutagenesis (oligo 1)

↓

FDTPs/Bs

~

in vitro mutagenesis (oligo 2)

↓

FDTPs/Xh

(1653)  
 Oligo 1 ACA ACT TTC AAC AGT TGA GGA GAC GGT GAC CGT AAG CTT CTG CAG TTG GAC CTG AGC  
 GGA GTG AGA ATA (1620)  
 (1653)  
 Oligo 2 ACA ACT TTC AAC AGT TTC CCG TTT GAT CTC GAG CTC CTG CAG TTG GAC CTG  
 (1704)  
 Oligo 3 GTC GTC TTT CCA GAC GTT AGT

GENE III

Fig.4 (i).

SIGNAL  
CLEAVAGE SITE

Fig.4 (ii).

(1624)  
 A TCT CAC TCC GCT  
 (1650)  
 GAA ACT GTT GAA AGT  
 Q V Q L Q V T V S S  
 B TCT CAC TCC GCT CAG GTC CAA CTG CAG AAG CTT ACG GTC ACC GTC TCC TCA ACT GTT GAA AGT  
 PstI BstEII  
 Q V Q L Q L E I K R  
 C TCT CAC TCC GCT CAG GTC CAA CTG CAG GAG CTC GAG ATC AAA CGG GAA ACT GTT GAA AGT  
 PstI XhoI

SphI

Pst I

130

190

250

310

370

430

490

SacI

Fig.5 (Cont).

E T V T I T C R A S G N I H N Y L A W Y  
GAAACTGTCACCATCACATGTCGAGCAAGTGGGAATATTCACAATTATTTAGCATGGTAT  
550 560 570 580 590 600

Q Q K Q G K S P Q L L V Y Y T T T L A D  
CAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATTATACAACAACCTTAGCAGAT  
610 620 630 640 650 660

VKD1.3

G V P S R F S G S G S G T Q Y S L K I N  
GGTGTGCCATCAAGGTTTCAGTGGCAGTGGATCAGGAACACAATATTTCTCTCAAGATCAAC  
670 680 690 700 710 720

S L Q P E D F G S Y Y C Q H F W S T P R  
AGCCTGCAACCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTGGAGTACTCCTCGG  
730 740 750 760 770 780

Myc Tag (TAG1)

T F G G G T K L E I K R E O K L I S E E  
ACGTTCCGGTGGAGGGACCAAGCTCGAGATCAAACGGGAACAAAACTCATCTCAGAAGAG  
790 800 810 820 830 840

XhoI

D L N \* \*

GATCTGAATTAATAATGATCAAACGGTAATAAGGATCCAGCTCGAATTC  
850 860 870 880

EcoRI

666101-6247460

Fig.6.

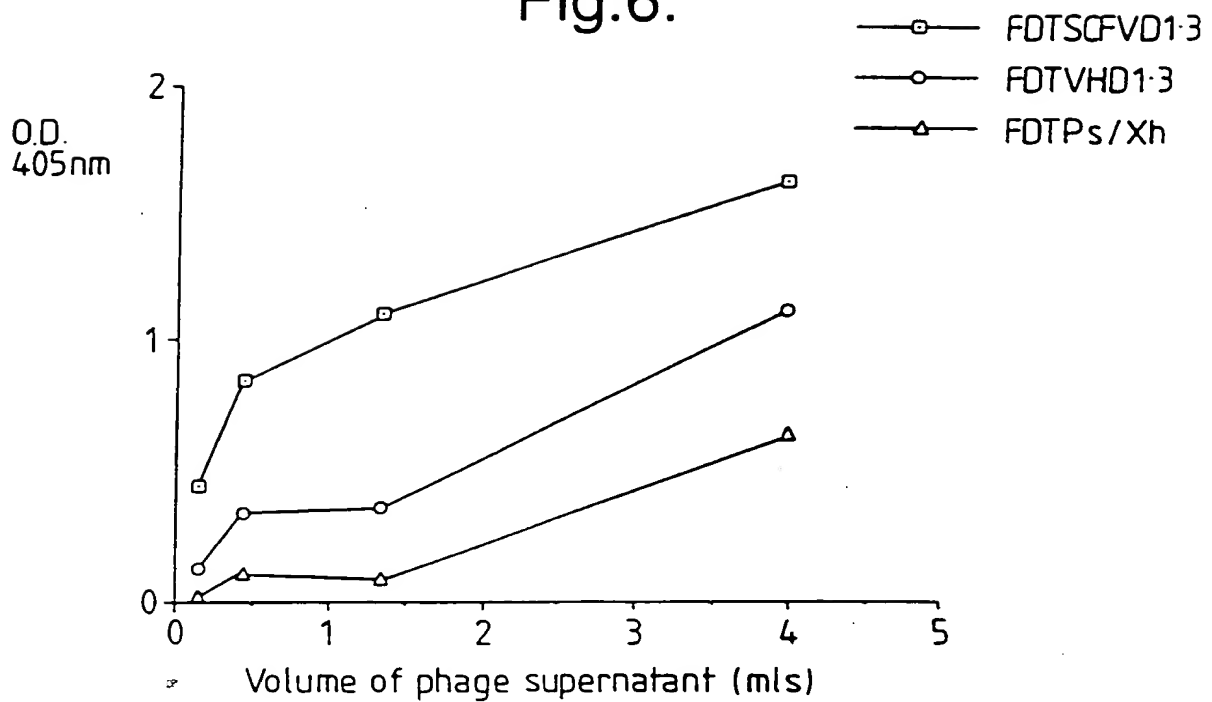


Fig.7.

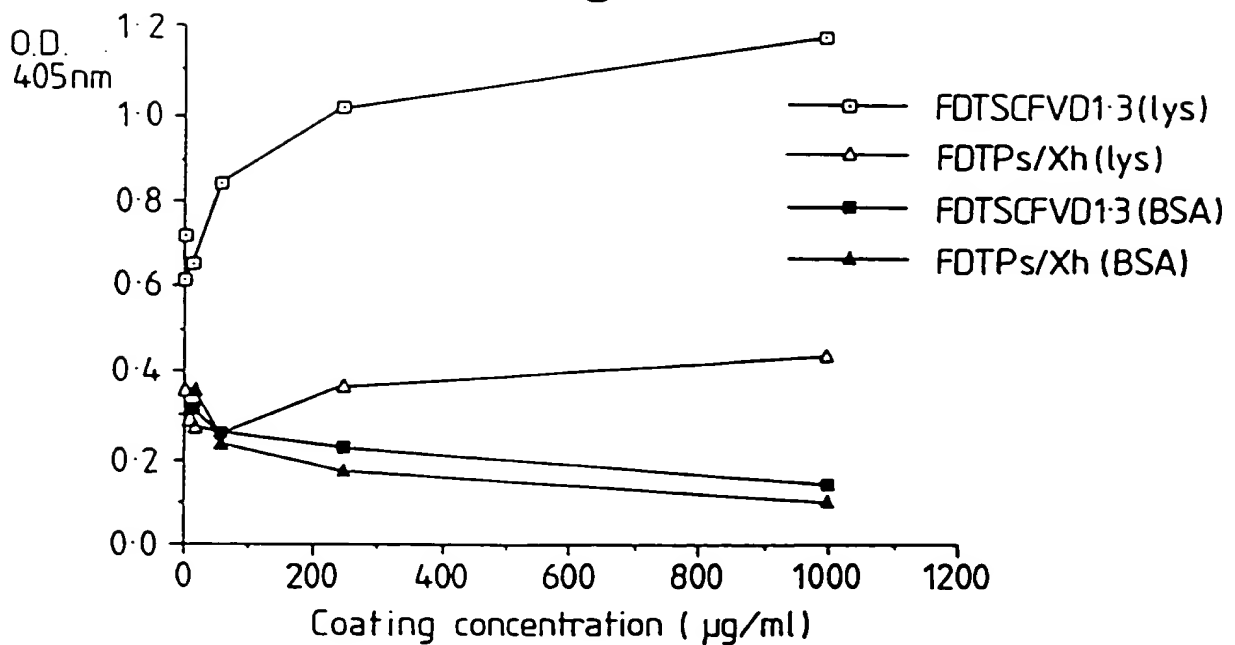


Fig.8.

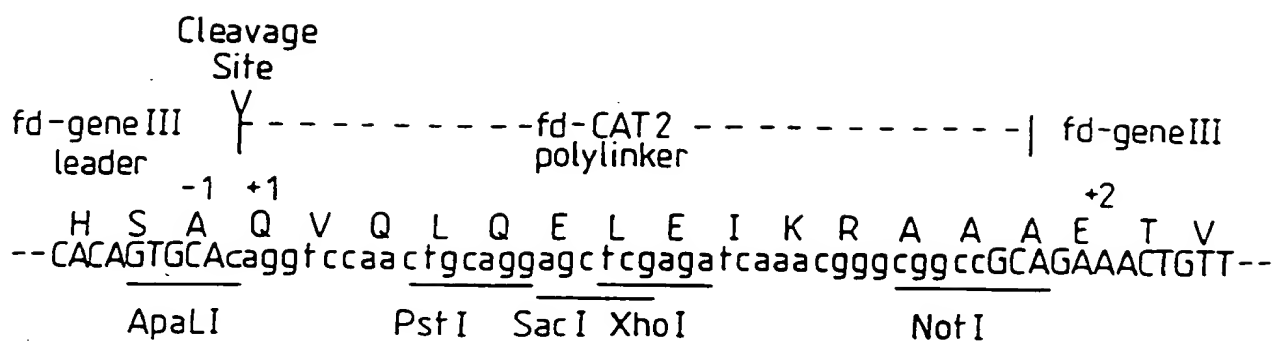
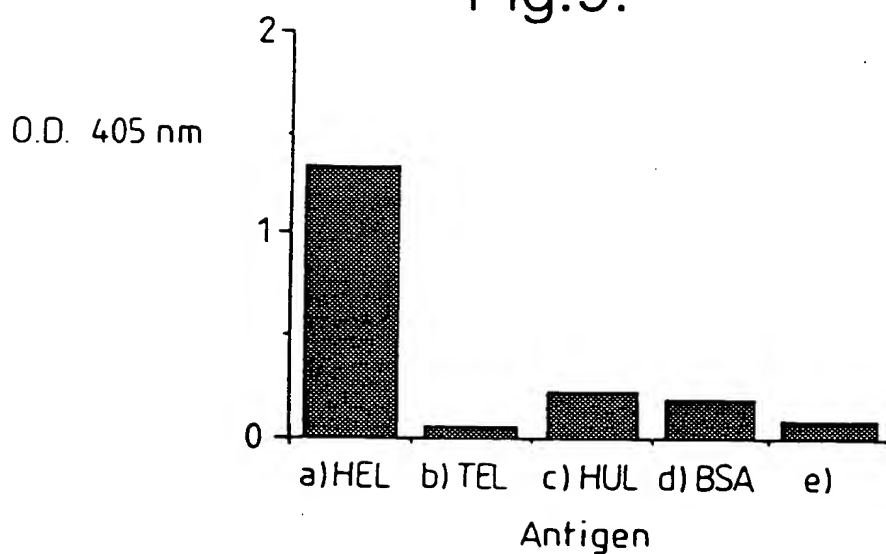


Fig.9.





M K Y L L P T A A

A G L L L A A Q P A M A Q V Q L Q E S  
GCTGGATTGTTATTACTCGCTGCCCACCCAGCGATGGCCCCAGGIGCAGCTGCAGGAGTCA  
70 80 90 100 110 120

S L T G Y G V N W V R Q P P G K G L E W  
TCATTAACCGGCTATGGTGTAACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGG  
190 200 210 220 230 240

S I S K D N S K S Q V F L K M N S L H T  
AGCATCAGCAAGGACAACCTCCAAGAGCCAAAGTTTTCTTAAAAATGAACAGTCTGCACACT  
310 320 330 340 350 360

D D T A R Y Y C A R E R D Y R L D Y W G  
GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC  
370 380 390 400 410 420

Q G T T V T V S S A S T K G P S V F P L  
CAAGGCACCAAGGTCAACGGTCTCTCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG  
430 440 450 460 470 480

A P S S K S T S G G T A A L G C L V K D  
GCACCCCTCCTCCAGAGCACCTCTGGGGGCACAGGGCCCTGGGCTGCCTGGTCAAGGAC  
490 500 510 520 530 540

[illegible]

Fig.10 (Cont 1).

Y F P E P V T V S W N S G A L T S G V H  
TACTTCCCCGAACCGGTGACGGTGTCTGTGGAACTCAGGCGCCCTGACCAAGCGGCGTGCAC  
550 560 570 580 590 600

T F P A V L Q S S G L Y S L S S V V T V  
ACCTTCCCGGTGTCTTACAGTCTCTAGGACTCTACTCCCTCAGCAGCGTGGTGAACGTG  
610 620 630 640 650 660

P S S S L G T Q T Y I C N V N H K P S N  
CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAAGCAAC  
670 680 690 700 710 720

T K V D K K V E P K S S \* \*  
ACCAAGGTGACAAGAAAGTTGAGCCCAATCTTCATAATAACCCGGGAGCTTGCATGCA  
730 740 750 760 770 780

M K Y L L P T A A A G L  
AATTCTATTTCAAGGAGACAGTCAATGAATAACCTATTGCCTACGGCAGCCGCTGGAT  
790 800 810 820 830 840

L L L A A Q P A M A D I E L T Q S P A S  
TGTTATTACTCGCTGCCCCAACCAAGCATGGCCGACATCGAGCTCACCCAGTCTCCAGCCT  
850 860 870 880 890 900

L S A S V G E T V T I T C R A S G N I H  
CCCTTCTGCGTCTGTGGGAGAACTGTACCATCACATGTGAGCAAGTGGGAATATT  
910 920 930 940 950 960

N Y L A W Y Q Q K Q G K S P Q L L V Y Y  
ACAATTATTAGCATGGTATCAGCAGAAACAGGGAAATCTCTCTAGCTCCTGGTCTATT  
970 980 990 1000 1010 1020

600 590 580 570 560 550 540 530 520 510 500 490 480 470 460 450 440 430 420 410 400 390 380 370 360 350 340 330 320 310 300 290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

Fig.10 (Cont 2).

T T T L A D G V P S R F S G S G S G T Q  
ATACAACAACCTTAGCAGATGGTGTGCCATCAAGGTTTCAGTGGCAGTGGATCAGGAACAC  
1030 1040 1050 1060 1070 1080

Y S L K I N S L Q P E D F G S Y Y C Q H  
AATATTCTCTCAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTAATGTCAC  
1090 1100 1110 1120 1130 1140

F W S T P R T F G G G T K L E I K R T V  
ATTTTGGAGTACTCTCTGGACGTTGGGTGGAGGCCAAGCTCGAGATCAAACGGACTG  
1150 1160 1170 1180 1190 1200

A A P S V F I F P P S D E Q L K S G T A  
TGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGT  
1210 1220 1230 1240 1250 1260

S V V C L L N N F Y P R E A K V Q W K V  
CCTCTGTTGIGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGGAAGG  
1270 1280 1290 1300 1310 1320

D N A L Q S G N S Q E S V T E Q D S K D  
TGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGTGTTCACAGAGCAGGACAGCAAGG  
1330 1340 1350 1360 1370 1380

S T Y S L S S T L T L S K A D Y E K H K  
ACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACA  
1390 1400 1410 1420 1430 1440

V Y A C E V T H Q G L S S P V T K S F N  
AAGTCTACGCCTGCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCAAAAGAGCTTCA  
1450 1460 1470 1480 1490 1500

R G E S \* \*  
ACCGCGGAGAGTTCATAGTAAGAATTC  
1510 1520

66707-627460

Fig.10 (Cont 3).

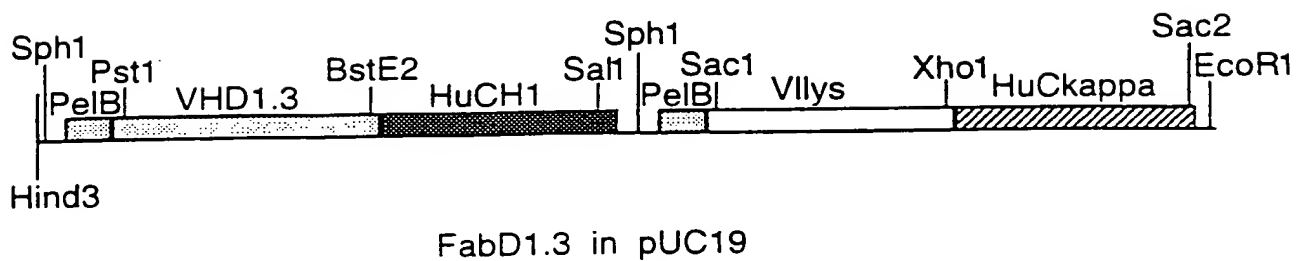
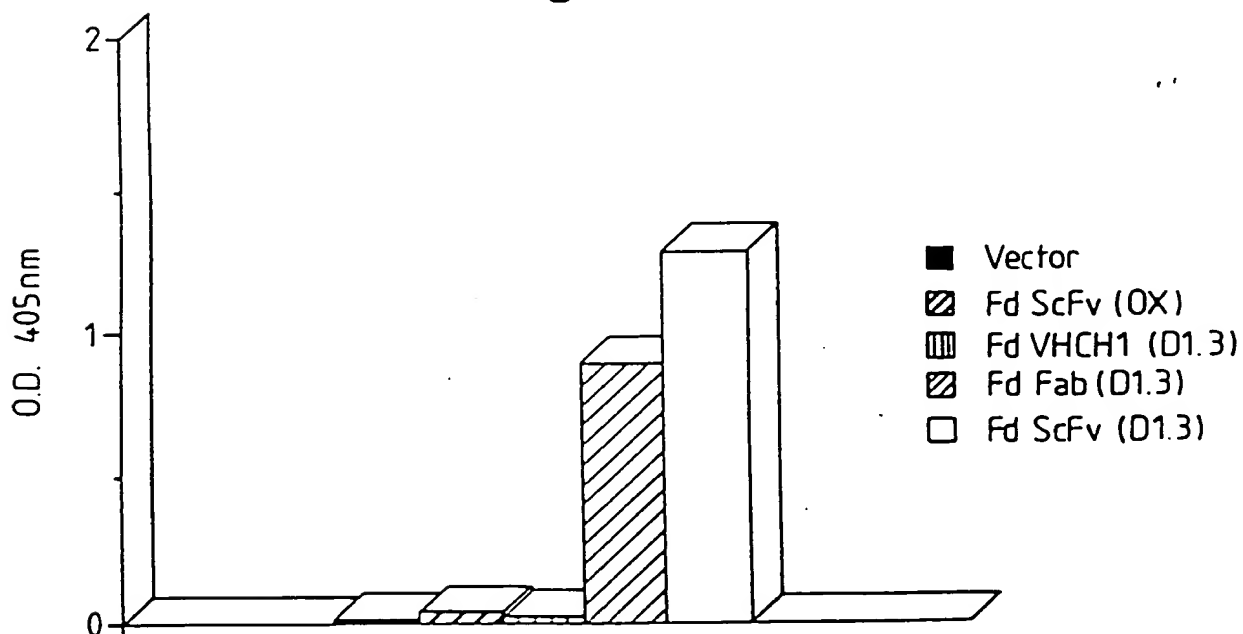



Fig.11.



[illegible]

[illegible]

Q V Q L Q E S G G L Q V Q P G G  
CAG GTG CAG CTG CAG GAG TCA GGA GGA GGC TTG GTA CAG CCT GGG GGT  
PstI  
S L R L S C A T S G F T F S N Y  
TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG TTC ACC TTC AGT AAT TAC  
Y M G W V R Q P P G K A L E W L  
TAC ATG GGC TGG GTC CGC CAG CCT CCA GGA AAG GCA CTT GAG TGG TTG  
G S V R N K V N G Y T T E Y S A  
GGT TCT GTT AGA AAC AAA GTT AAT GGT TAC ACA ACA GAG TAC AGT GCA  
S V K G R F T I S R D N F Q S I  
TCT GTG AAG GGG CGG TTC ACC ATC TCC AGA GAT AAT TTC CAA AGC ATC  
L Y L Q I N T L R T E D S A T Y  
CTC TAT CTT CAA ATA AAC ACC CTG AGA ACT GAG GAC AGT GCC ACT TAT  
Y C A R G Y D Y G A W F A Y W G  
TAC TGT GCA AGA GGC TAT GAT TAC GGG GCC TGG TTT GCT TAC TGG GGC  
Q G T L V T v s s g g g g s g g g g s  
CAA GGG ACC CTG GTC ACC gtc tcc tca ggtggaggcggttcaggcggagggtggcct  
BstEII  
g g g g s d i E L T Q T P L S L P V  
ggcggtggcggtatcgagac atc GAG CTC ACC CAA ACT CCA CTC TCC CTG CCT GTC  
SacI  
S L G D Q A S I S C R S S Q S I  
AGT CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT  
V H S N G N T Y L E W Y L Q K P  
GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA CCA  
PstI  
G Q S P K L L I Y K V S N R F S  
GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT  
G V P D R F S G S G S G T D F T  
GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCG GGG ACA GAT TTC ACA  
L K I S R V E A E D L G V Y Y C  
CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC  
F Q G S H V P Y T F G G G T K L  
TTT CAA GGT TCA CAT GTT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTC  
E I K R  
GAG ATC AAA CGG  
XhoI

Fig.14.

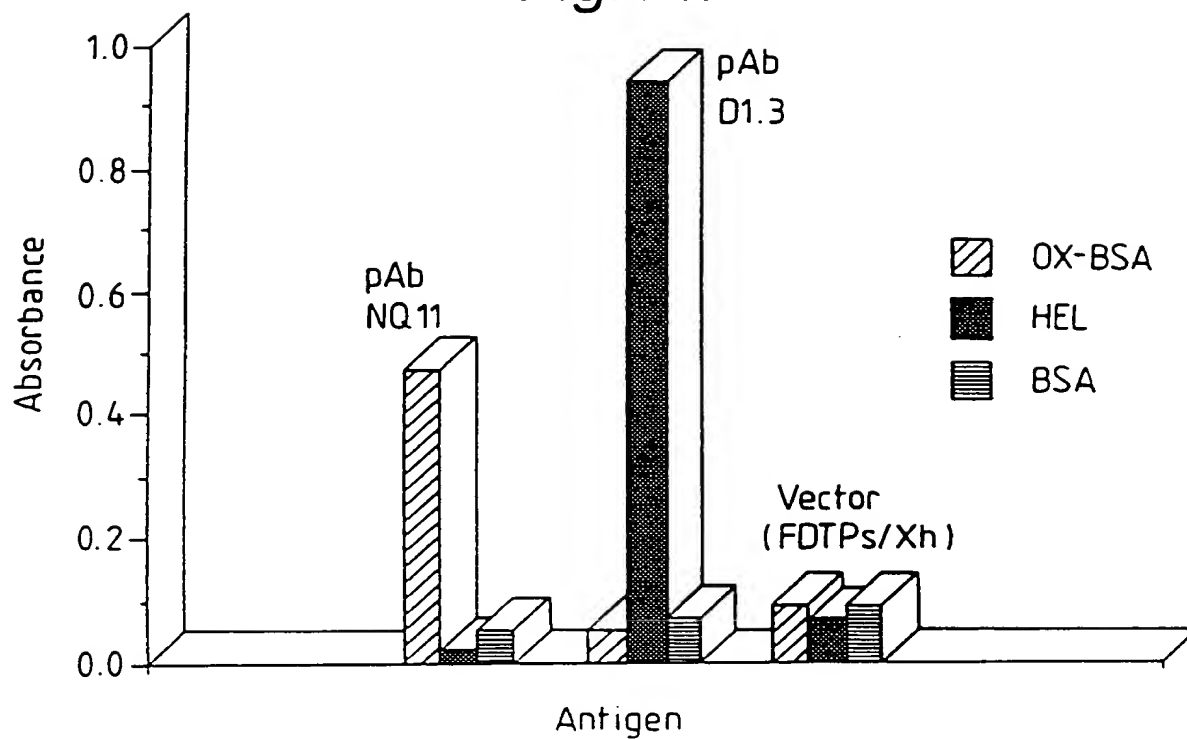


Fig.15.

5' END

TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG  
 ApaL1

3' END

K A A L G L K  
 AAA GCC GCT CTG GGG CTG AAA GCG GCC GCA GAA ACT GTT GAA AGT etc.  
 Not I

Fig.16 (i).

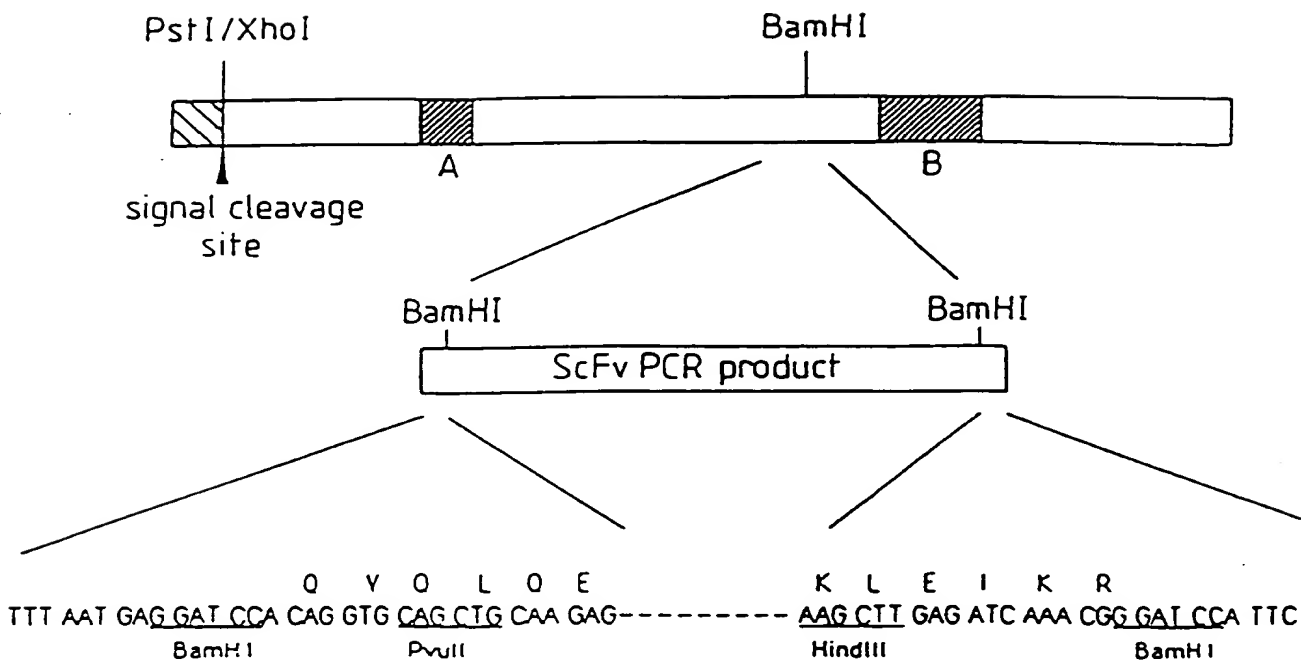


Fig.16 (ii).

A (1834) 5' GAG GGT GGT GGC TCT  
 " " "C " "  
 " " "C " "  
 " " "C " ACT 3'(1839)

B (2284) 5' - GGC GGC GGC TCT  
 - GGT GGT GGT -  
 - GGC GGC -  
 GAG - GGC -  
 " - GGT -  
 - GGC -  
 - GGT -  
 - GGC - 3'(2379)

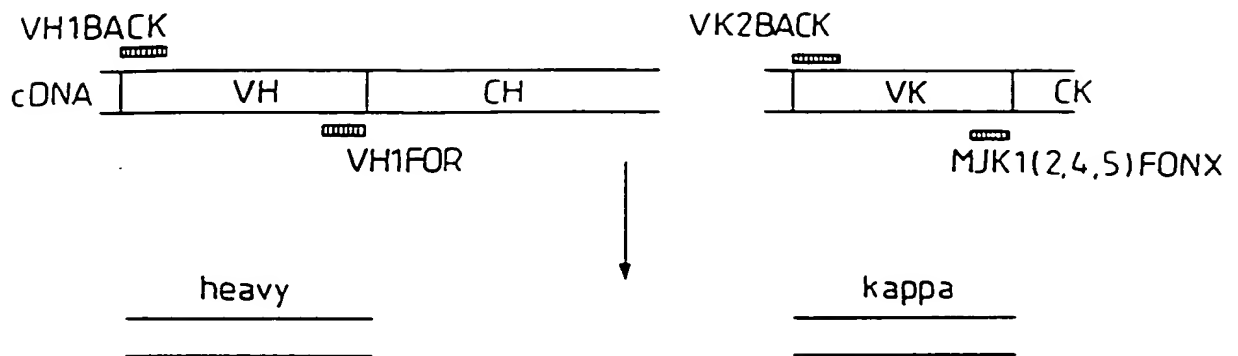
Reverse complement of mutagenic  
 oligo G38amlink

5' GAG GGT GGC GGA TCC  
 T  
 GAG GGT GGC GG 3'

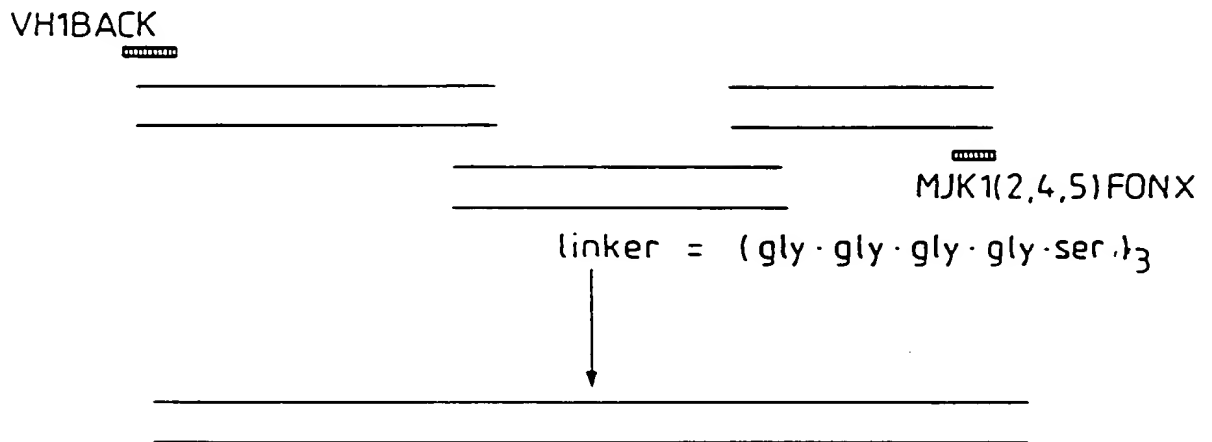


Fig.17.

1) PRIMARY PCR



2) ASSEMBLY PCR



3) ADDING RESTRICTION SITES

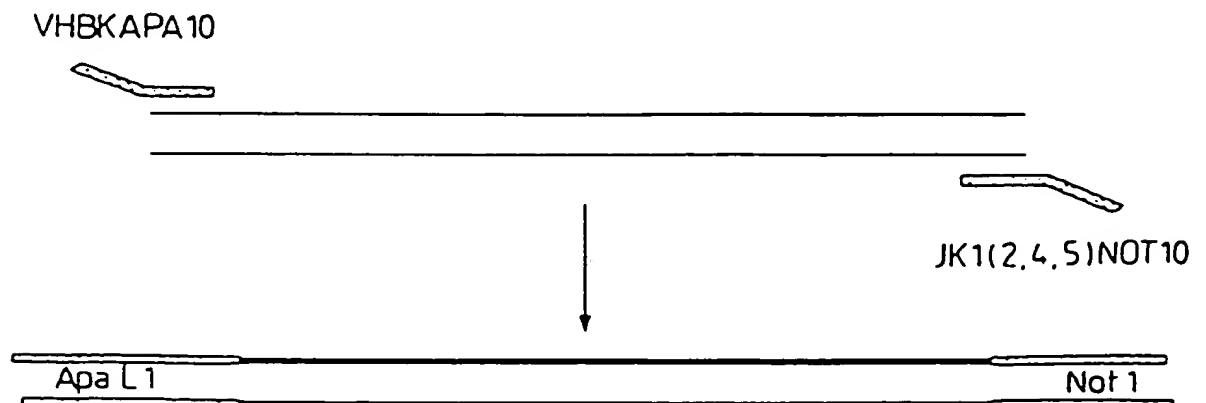


Fig.18.

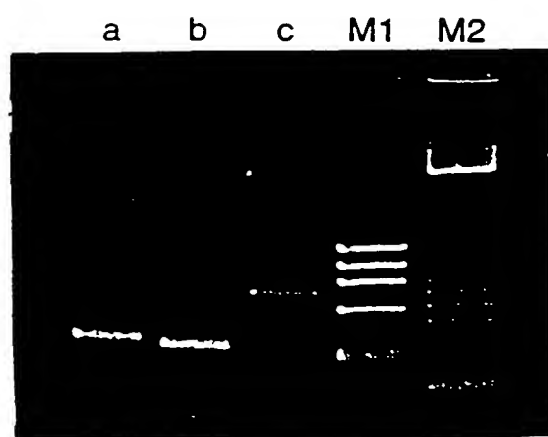


Fig.19.

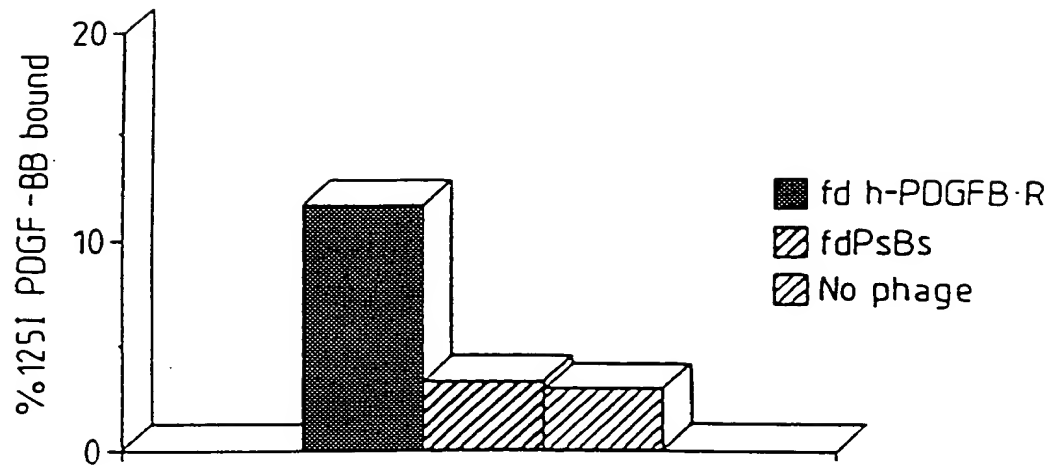
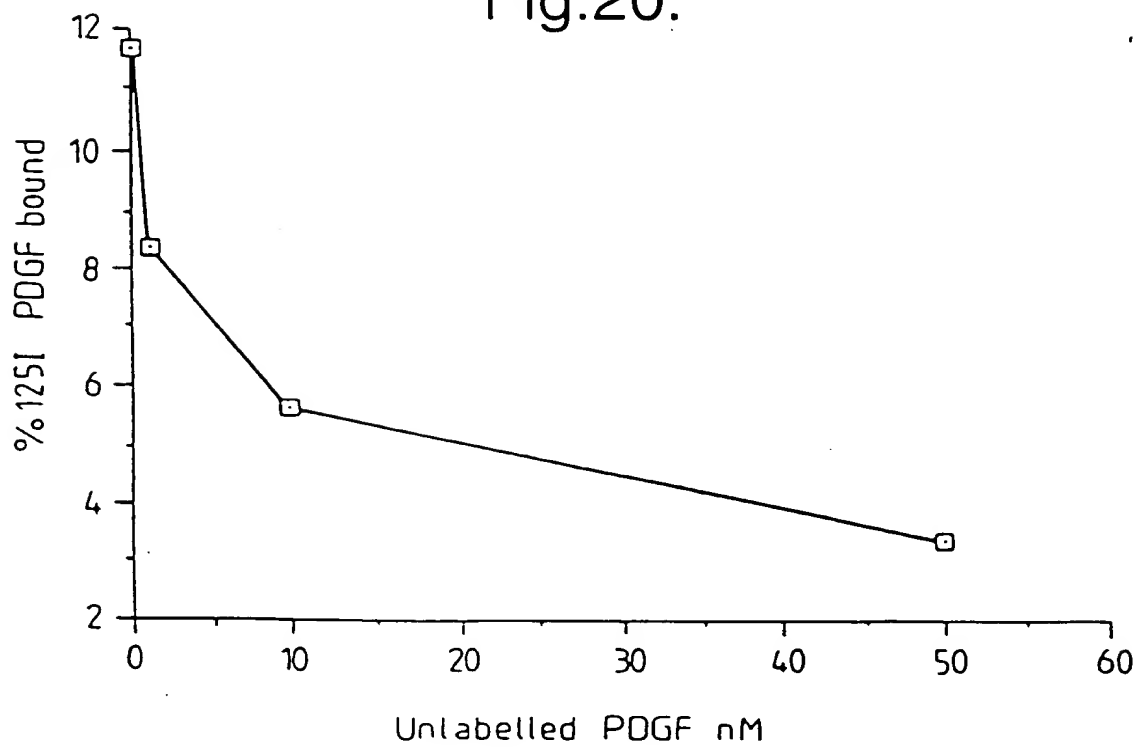


Fig.20.



666701 6442460

Fig.21.

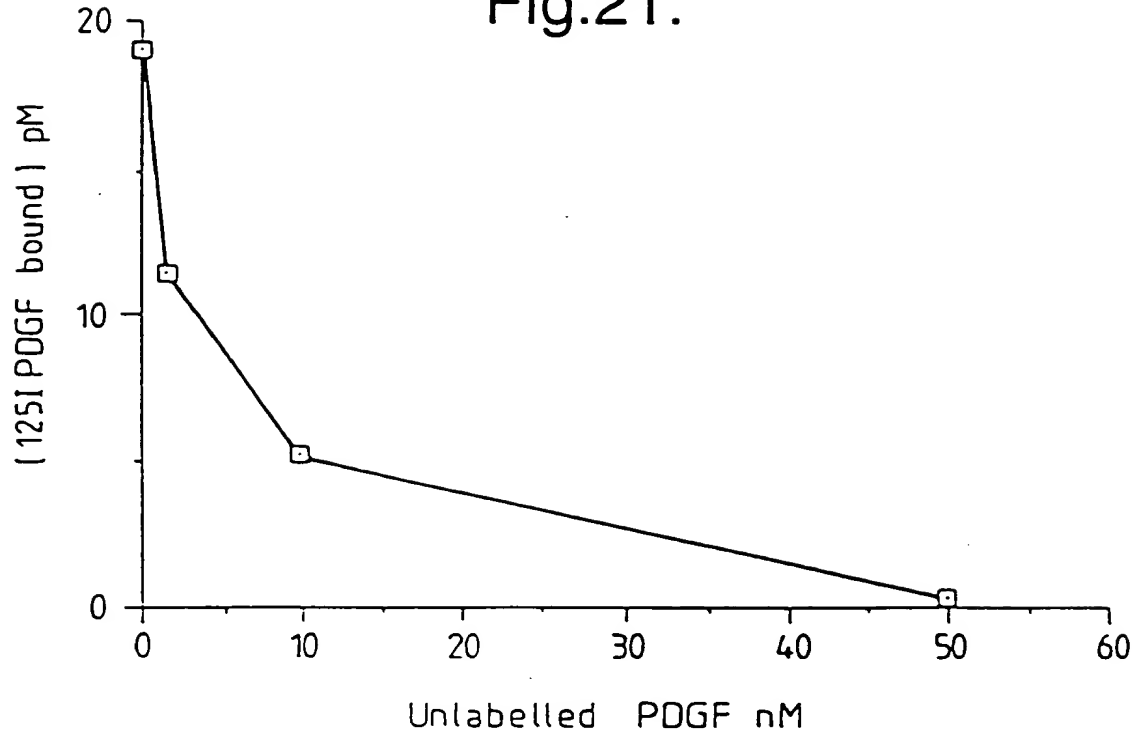


Fig.22.

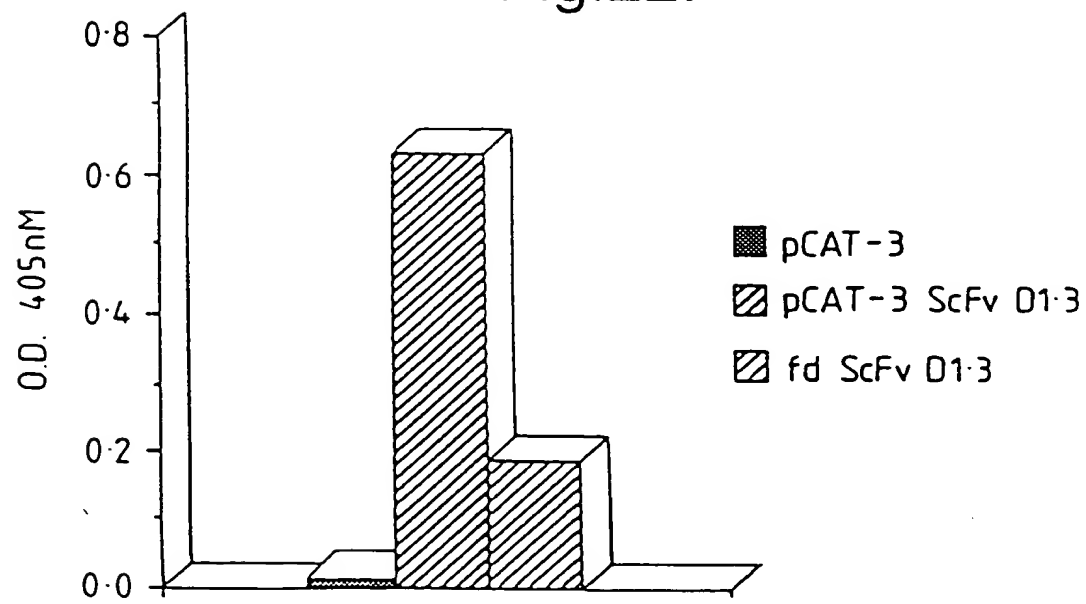


Fig.23( i )

d  
M

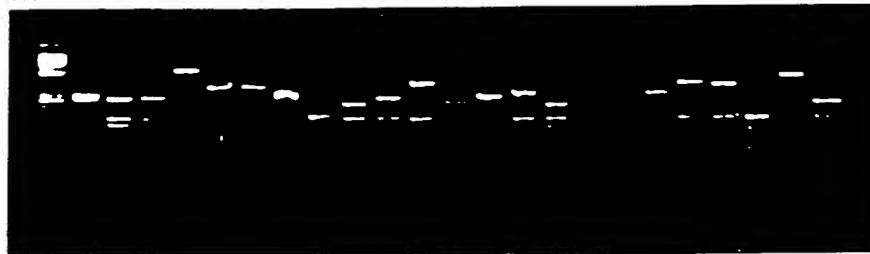


Fig.23(ii)

M



Fig. 24.

## VH sequences

**from combinatorial library:**

[illegible]

Ir m hierarchial library VH-rep x Vκ-d:

[illegible]

Fig.24 (Cont).

Vk sequences

from combinatorial library:

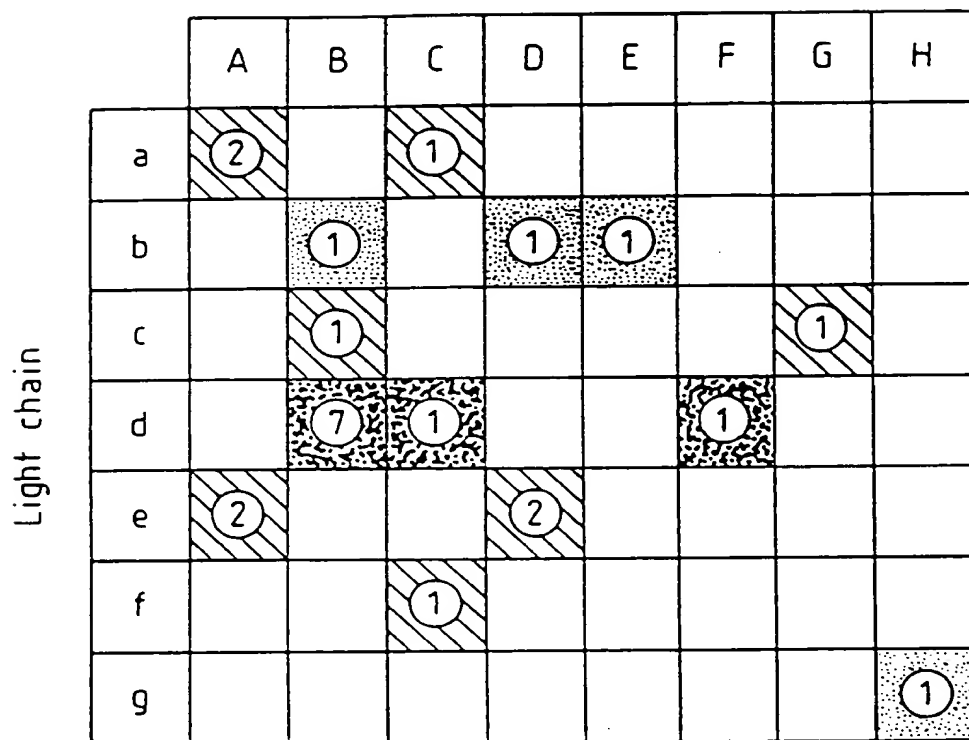
	COR1	COR2	COR3	
a	DIELTQSPSPSLASLGERVSLTC	RASQELSCVLS	WLOQKPDGSIKRLIY	AASTLAS
b	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
c	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
d	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
e	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
f	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
g	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS

from hierarchical library VH-B x Vκ-rep:

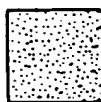
	COR1	COR2	COR3	
h	DIELTQSPSPSLASLGERVSLTC	RASQELSCVLS	WLOQKPDGSIKRLIY	AASTLAS
i	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
j	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
k	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
l	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
m	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
n	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
o	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
p	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
q	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
r	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
s	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
t	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
u	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
v	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS
w	DIELTQSPSPSLASLGERVSLTC	RASSSV66SYLH	WLOQKPDGSIKRLIY	AASTLAS

Fig.25.

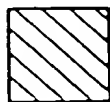
HEAVY CHAIN



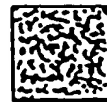
OD<sub>405nm</sub> in ELISA



0.2-0.9



0.9-2.0



>2.0



Fig.26(a).

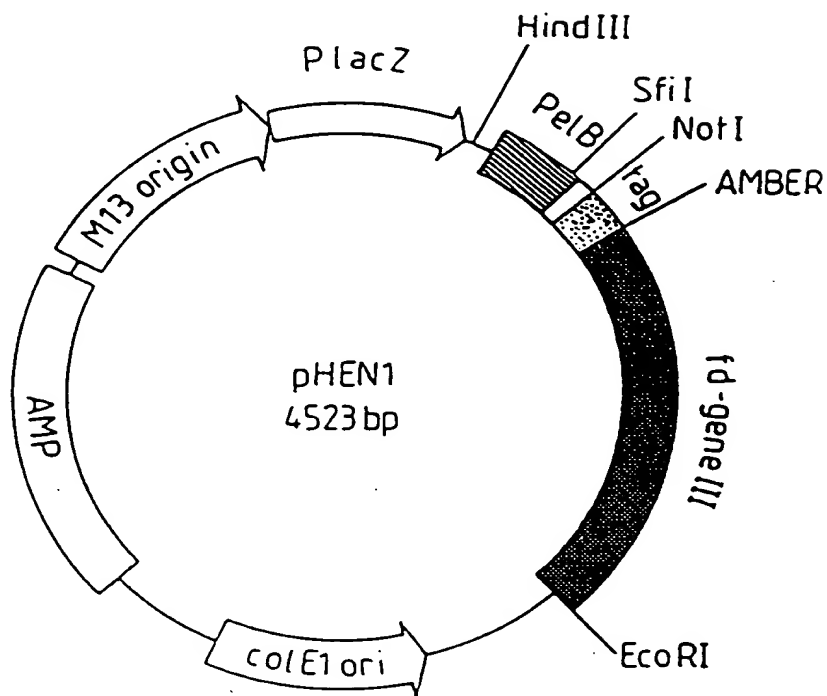


Fig.26(b).

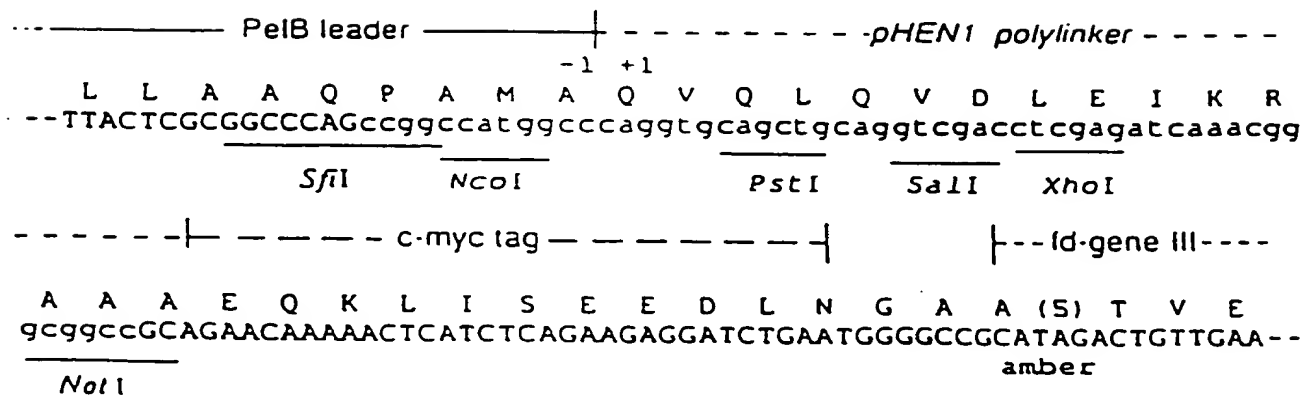


Fig.27.

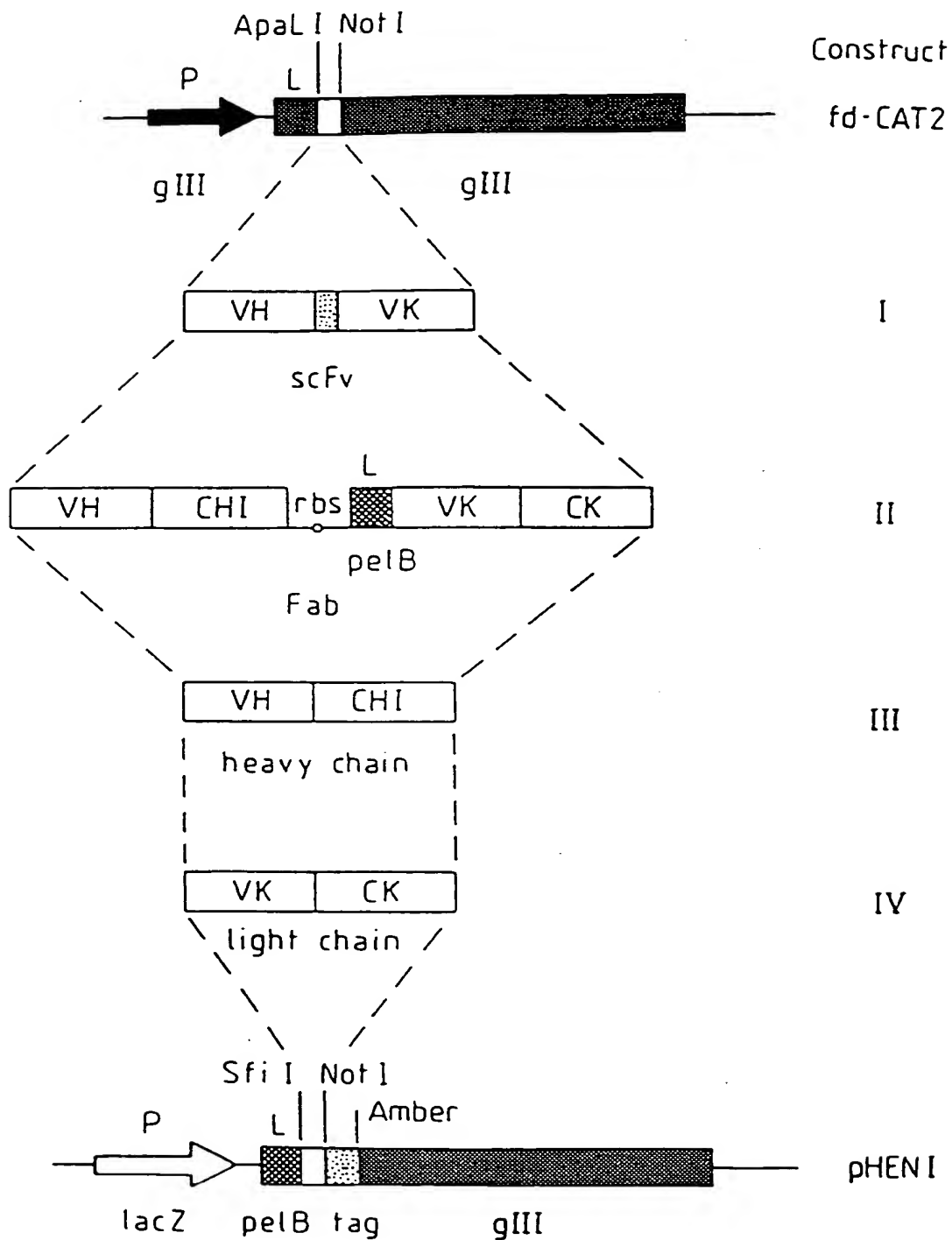


Fig.28.

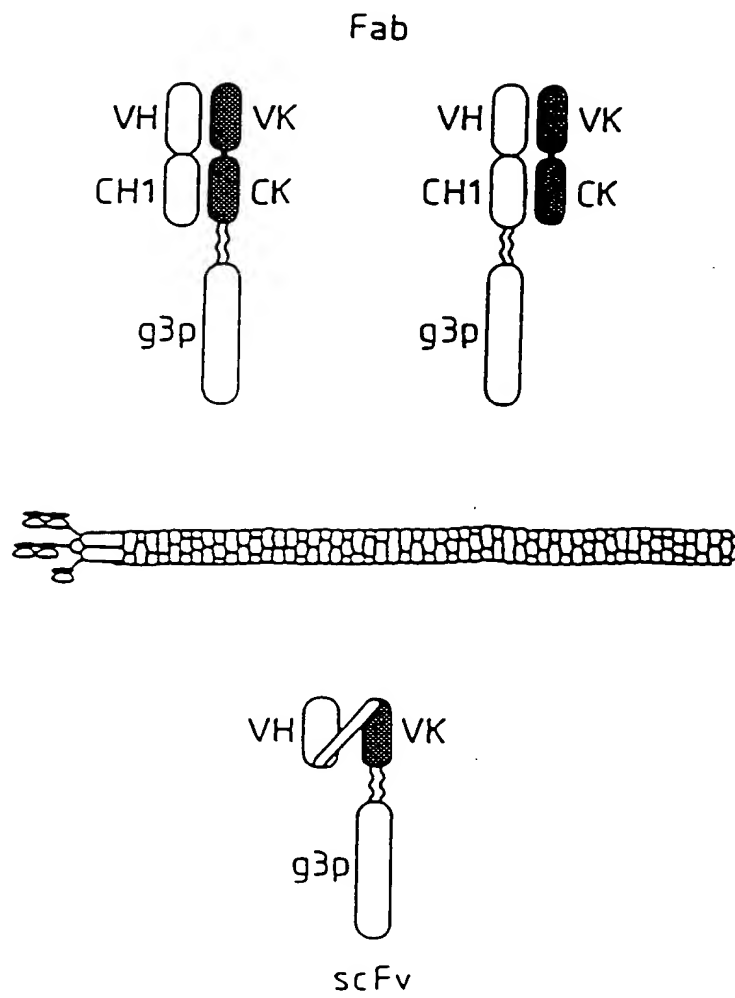


Fig.29.

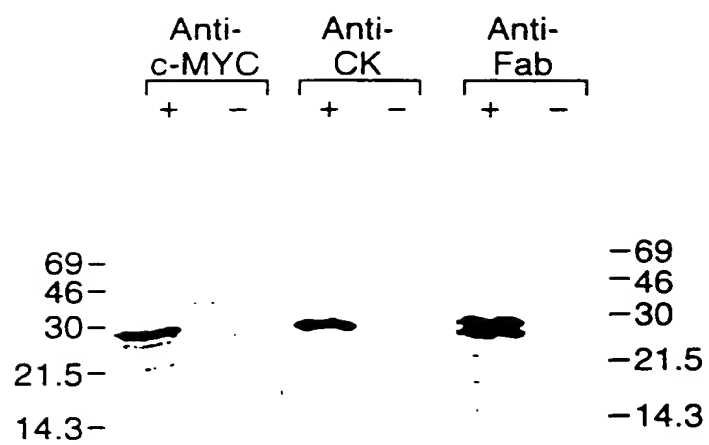


Fig.30.

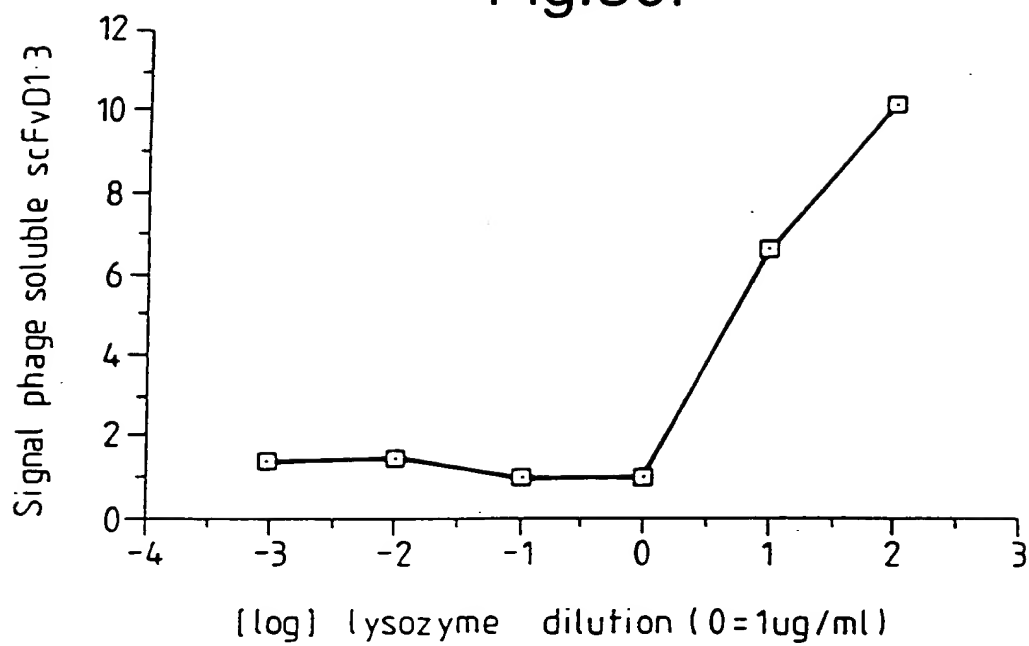


Fig.31.

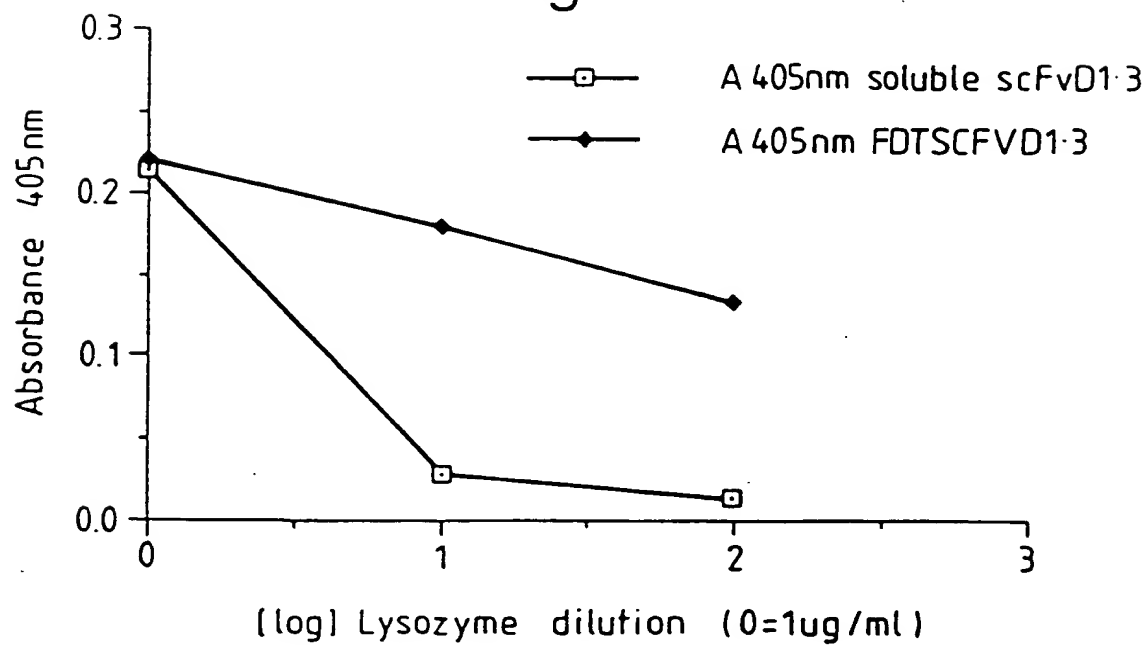


Fig.32.

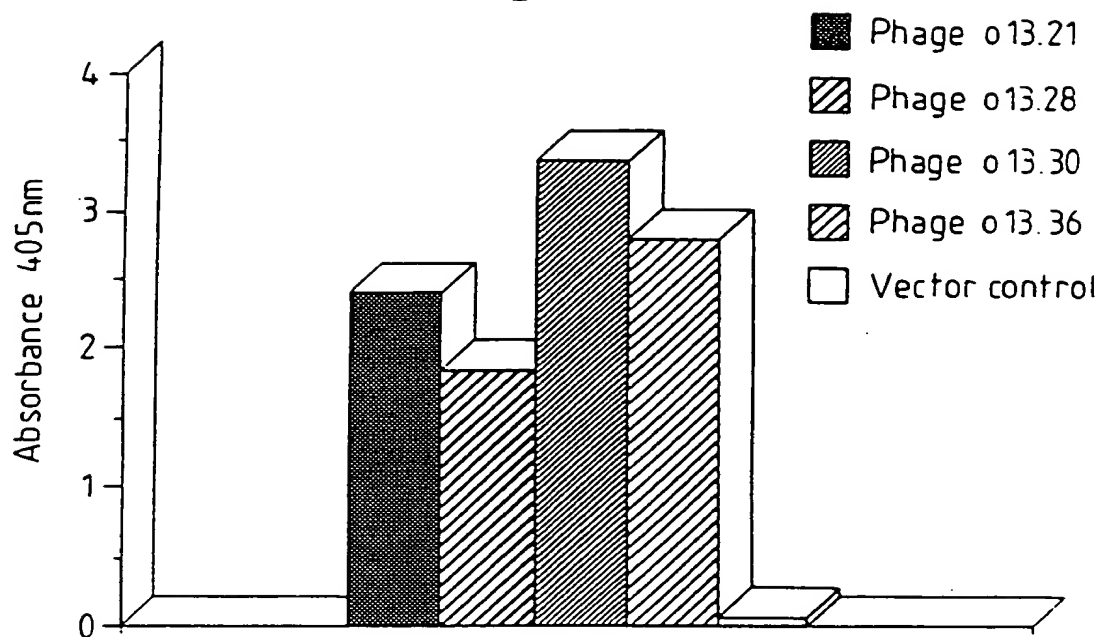


Fig.33.

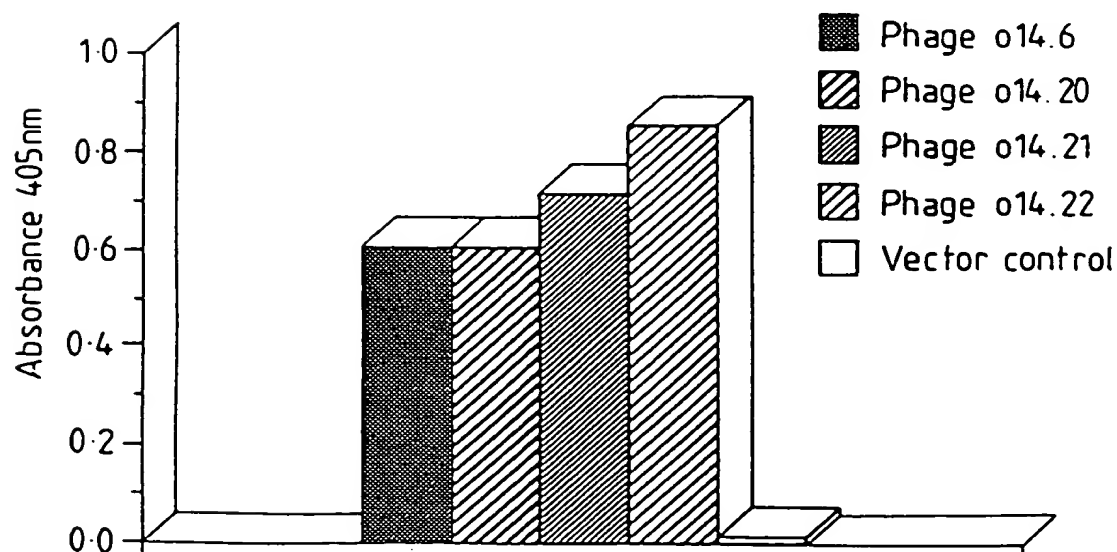


Fig.34.

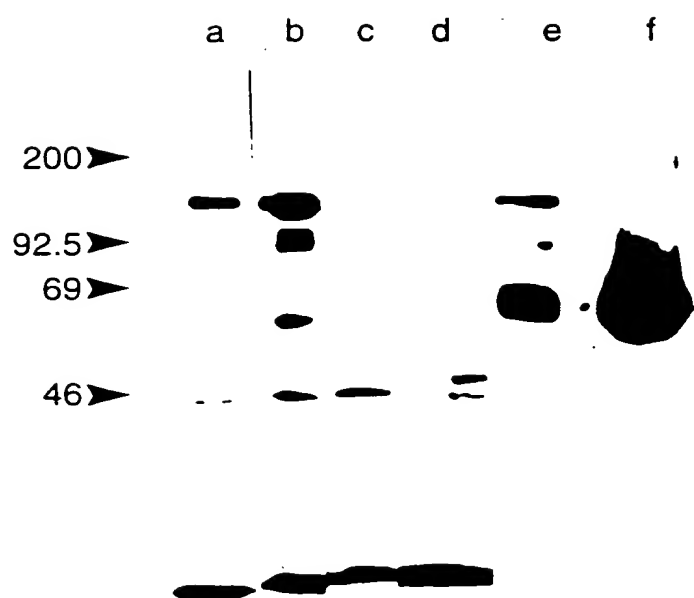


Fig.35A.

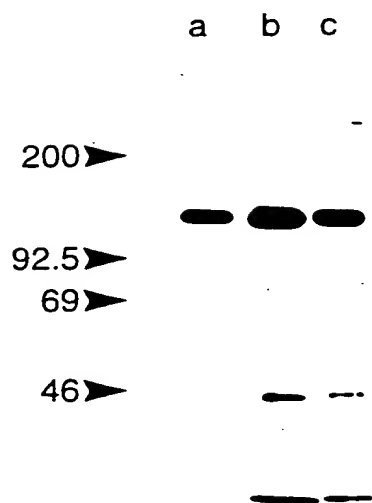


Fig.35B.

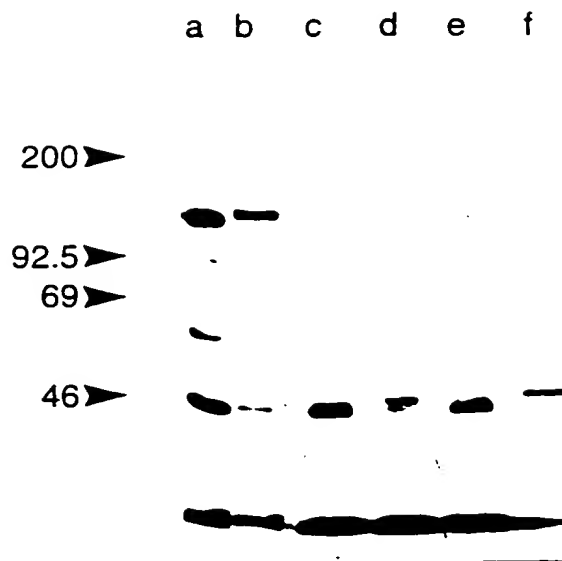


Fig.36.

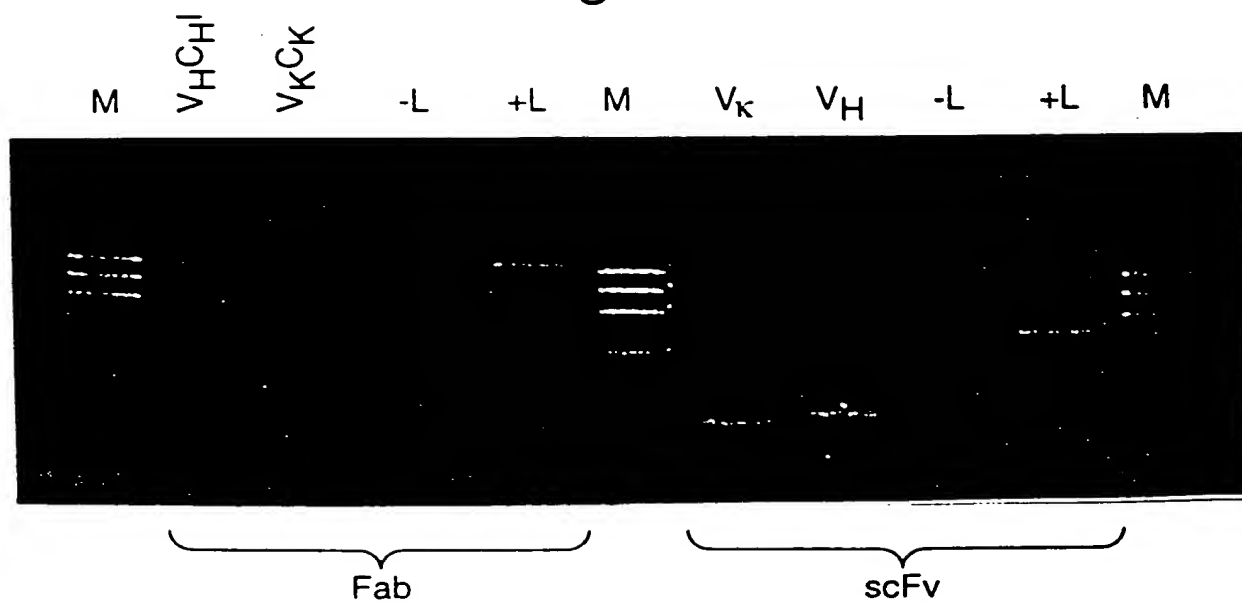
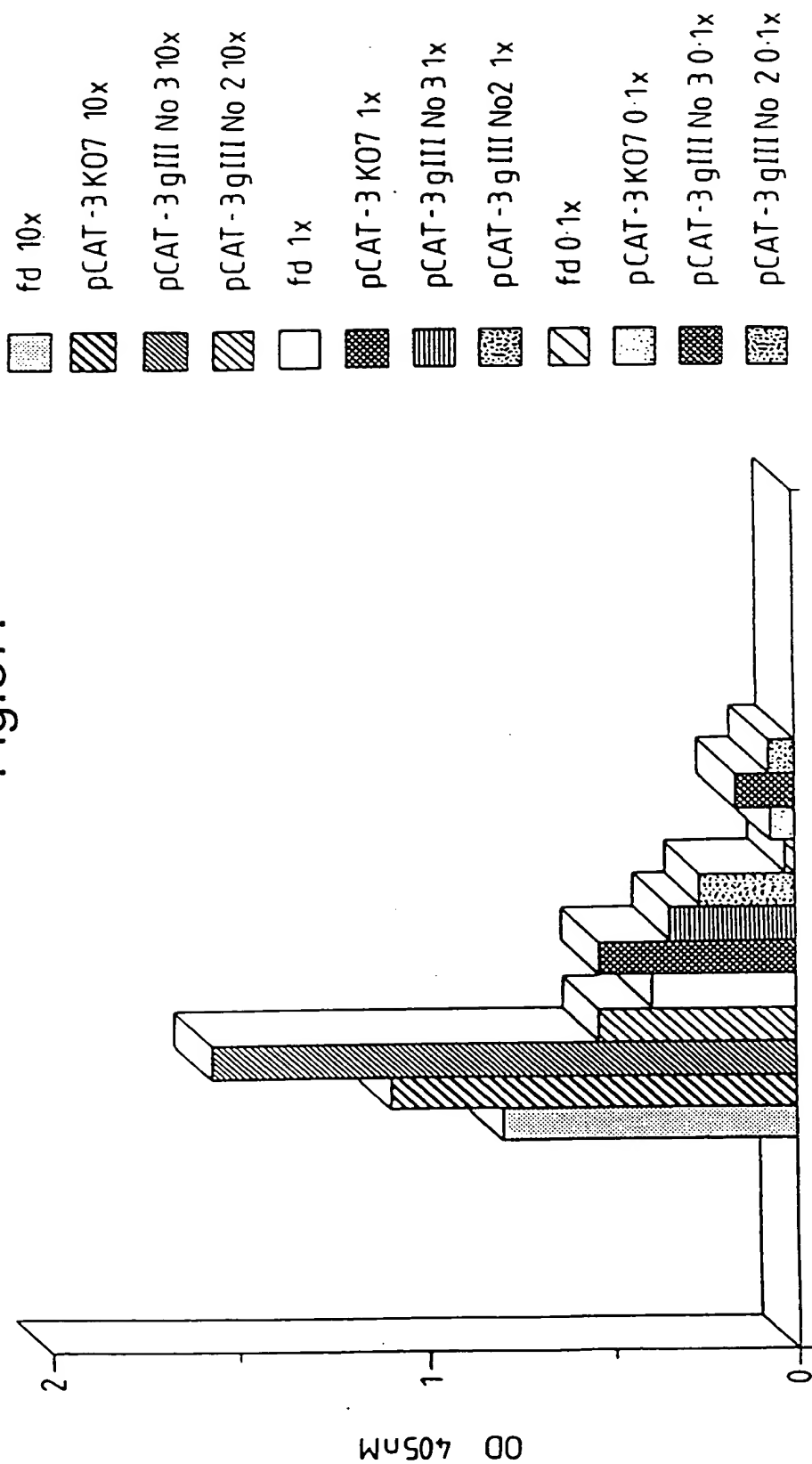




Fig.37.



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Fig.38A.



Fig.38B.

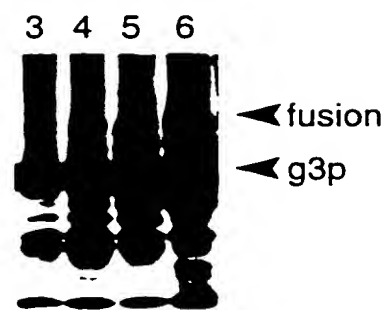


Fig.39.

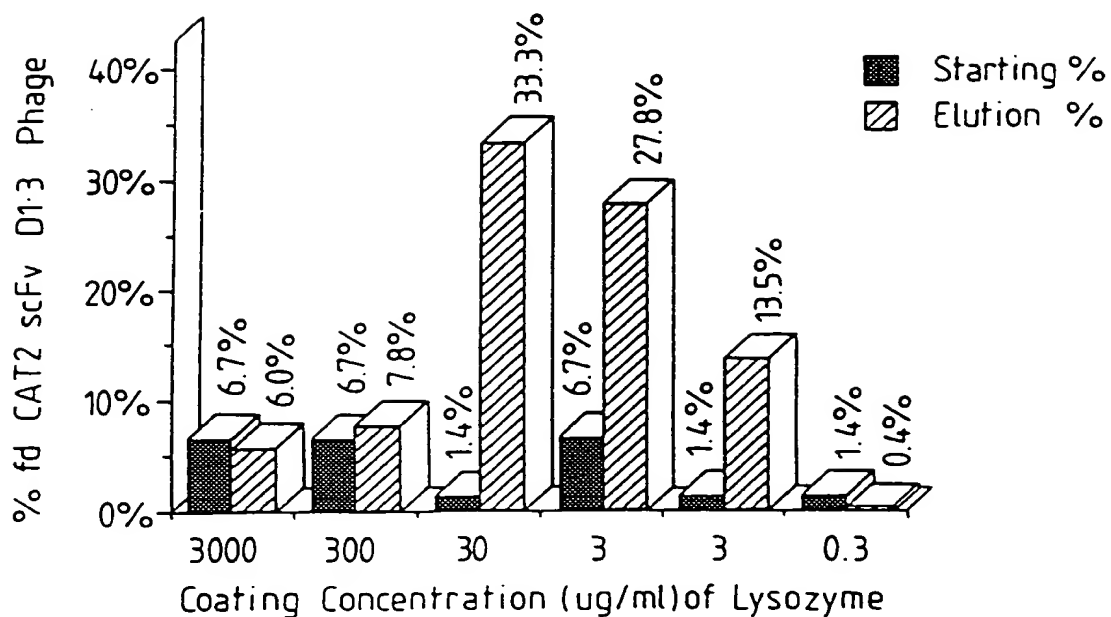


Fig.40.

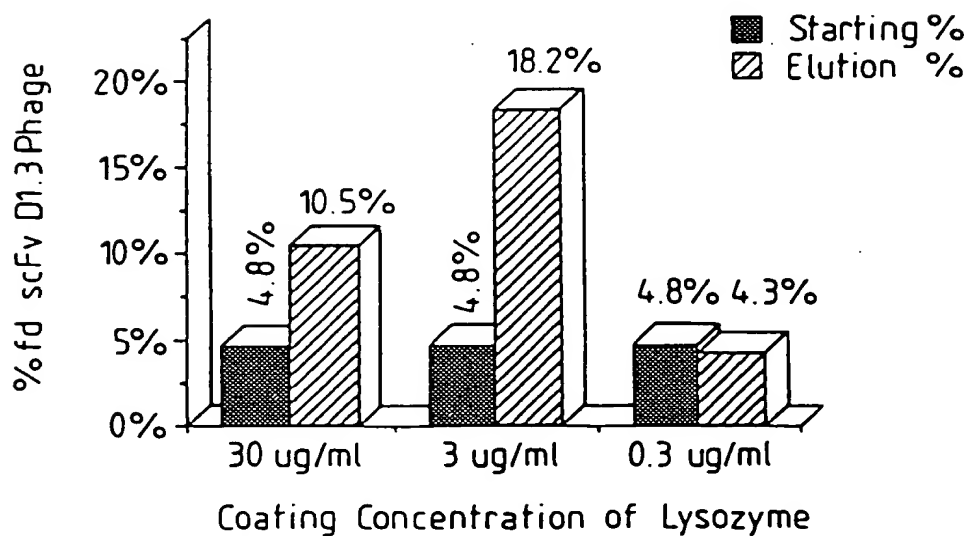


Fig.41.

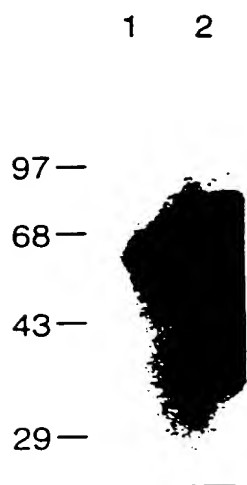


Fig.42.

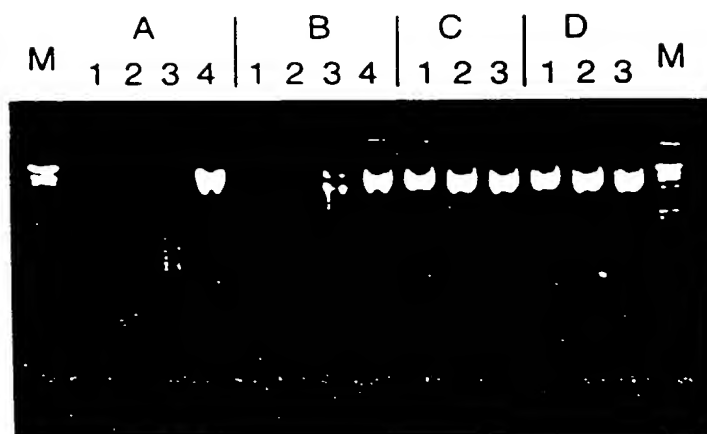


Fig.43.

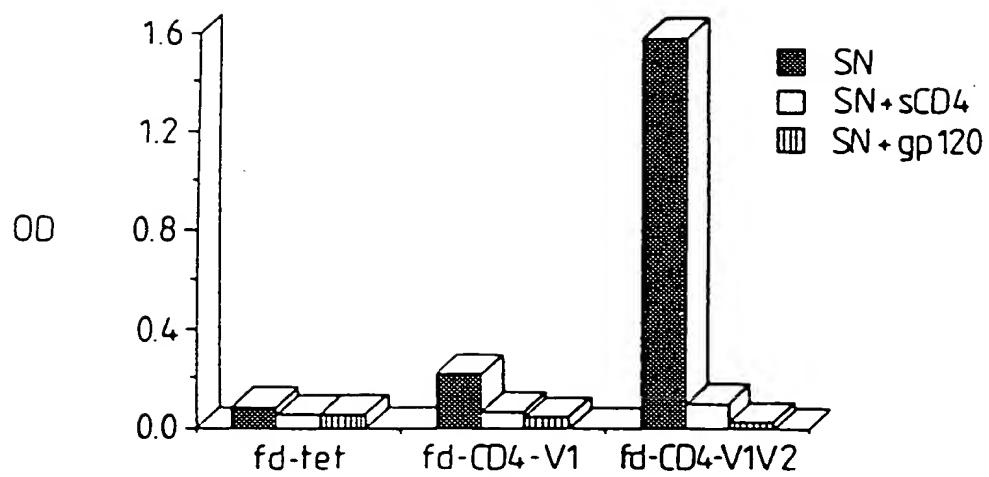


Fig.44 (i).

10	20	30	40	50	60	70	80	90
TTCTATTCTCACAGTGCACAGGTCCAGCTGCAGCAGTCTGGGGCTGAGCTTGTGAAGCCTGGGGCTTCAGTGAAGCTGTCTCCTGCAAGGCT								
AAGATAAGAGTGTACGTTGCCAGGTCCAGCTCAGACCCCGACTCGAACACTTCGGACCCCGAAGTCACCTTCGACAGACGTTCCGA								
PheTyrSerHisSerAlaGlnValGlnLeuGlnSerGlyAlaGluLeuValLysProGlyAlaSerValLysLeuSerCysLysAla								
100	110	120	130	140	150	160	170	180
TCTGGCTACACCTTACCCAGCTACTGGATGCACCTGGGTGAAGCAGAGGCCCTGGACGAGGCCCTTGAGTGGATTGGAAGGATTGATCCTAAT								
AGACCCGATGTGGAAGTGGTCGATGACCTACGTGACCCACTTCGTCTCCGGACCTGCTCCGGAACCTCACCTAACCTTCCCTAACTAGGATTA								
SerGlyTyrThrPheThrSerTyrTrpMetHisTrpValLysGlnArgProGlyArgGlyLeuGluTrpIleGlyArgIleAspProAsn								
190	200	210	220	230	240	250	260	270
AGTGGTGGTACTAAGTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAACCCCTCCAGCACAGCCTACATGCAGCTCAGC								
TCACCAACCATGATTCTTACTCTTCAAGTTCTCGTTCGGGTGACTGACATCTGTTTGGGAGGTCGTGTCGGATGTACGTCGAGTCG								
SerGlyGlyThrLysTyrAsnGluLysPheLysSerLysAlaThrLeuThrValAspLysProSerSerThrAlaTyrMetGlnLeuSer								
280	290	300	310	320	330	340	350	360
AGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTGCAAGNTACGACTACGGTAGTAGCTACTACTTTGACTACTGGGGCCAAAGGGACC								
TCGGACTGTAGACTCTCTGAGACGCCAGATAATAACACGTTCTATGCTGATGCCATCATCGATGATGAACACTGATGACCCCGGTTCCCTGG								
SerLeuThrSerGluAspSerAlaValTyrTyrCysAlaArgTyrAspTyrGlySerSerTyrTyrPheAspTyrTrpGlyGlnGlyThr								
370	380	390	400	410	420	430	440	450
ACGGTCAACCGTCTCCTCAGGTGAGGCGGTTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCCAGGCTGTTGGGACACAGGAATCTGCA								
TGCCAGTGGCAGAGGATCCACCTCCGCCAAGTCCGCCCTCCACCGAGACCGCCACCGCTAGGTCGACAAACCCCTGTGTCCTTAGACGT								
ThrValThrValSerSerGlyGlyGlySerGlyGlyGlySerGlyGlyGlySerGlnAlaValGlyThrGlnGluSerAla								
460	470	480	490	500	510	520	530	540
CTCACACATCACCTGGTGAAACAGTCACACTCACTTGTGCGCTCAAGTACTGGGGCTGTTACAACACTAGTAACATATGCCAAGTGGGTCCAA								
GAGTGGTGTAGTGACCACTTTGTGTCAGTGTGAGTGAACAGCGAGTTTCATGACCCCGACAAATGTTGATCATATTGATACGGTTGACCCAGGTT								
LeuThrThrSerProGlyGluThrValThrLeuThrCysArgSerSerThrGlyAlaValThrThrSerAsnTyrAlaAsnTrpValGln								
550	560	570	580	590	600	610	620	630
GAAAAACCAAGATCATTTTACTGGTCTAATAGGTGTACCAACACCGAGCTCCAGGTGTTCTCGCCAGATTCTCAGGCTCCCTGATT								
CTTTTGGTCTAGTAAATAAGTGACCAAGATTATCCACCATGGTTGTTGGCTCGAGGTCCACAAAGGACCGTCTAAGAGTCCGAGGGACTAA								
GluLysProAspHisLeuPheThrGlyLeuIleGlyGlyThrAsnAsnArgAlaProGlyValProAlaArgPheSerGlySerLeuIle								

Fig. 44 (ii).

Fig.45.

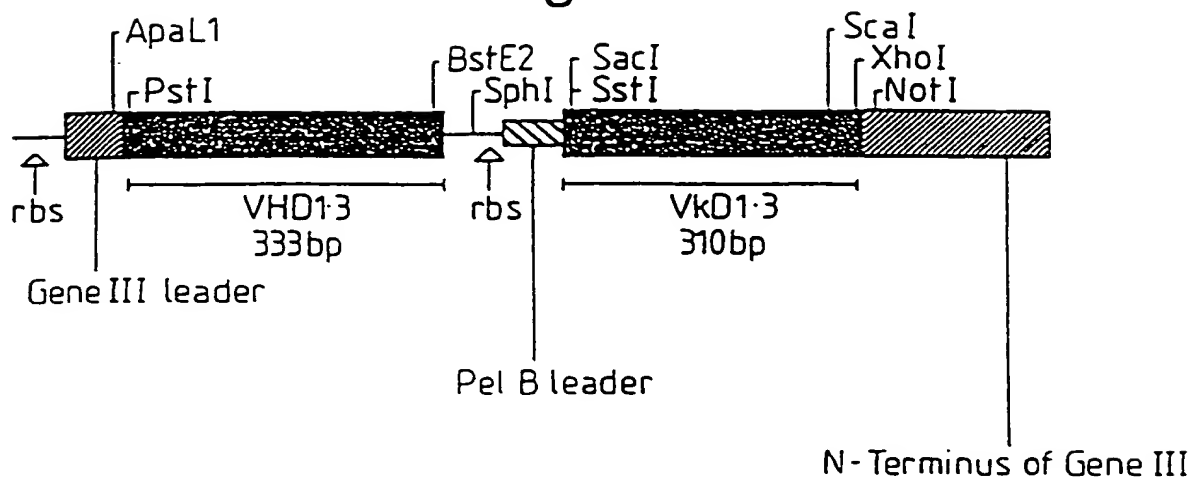


Fig.46.

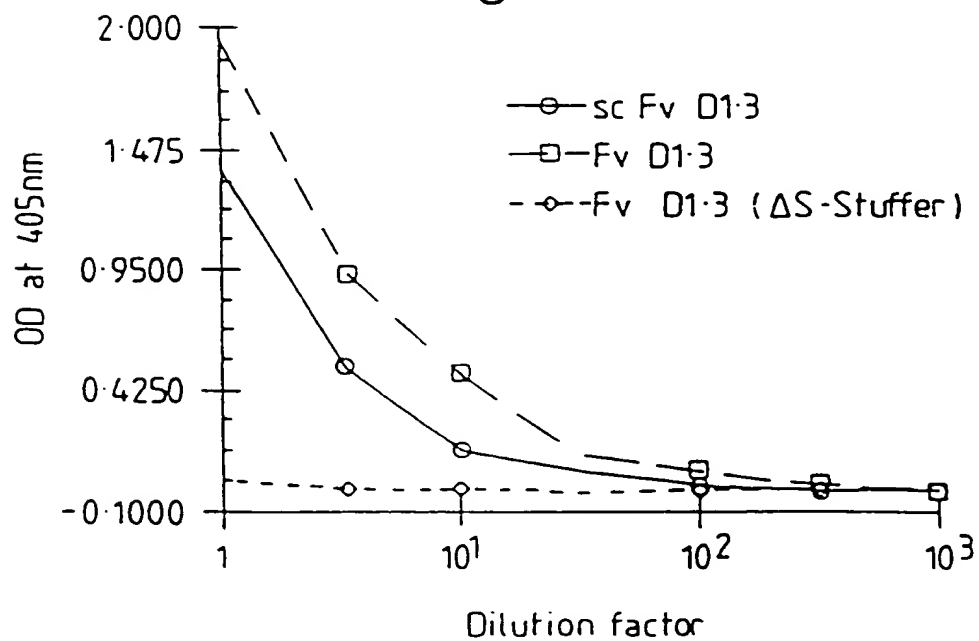




Fig.47.

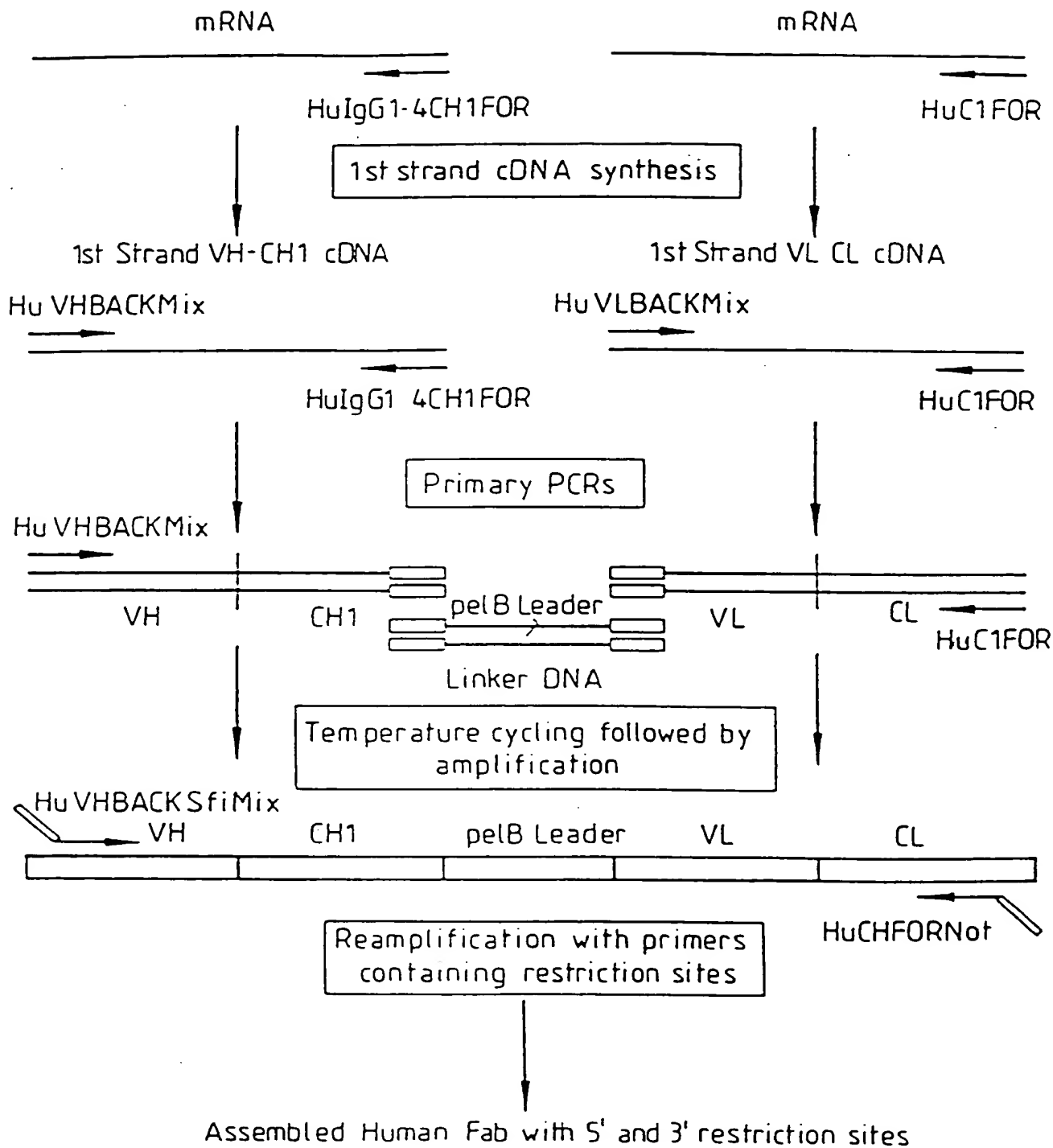


Fig.48(i)

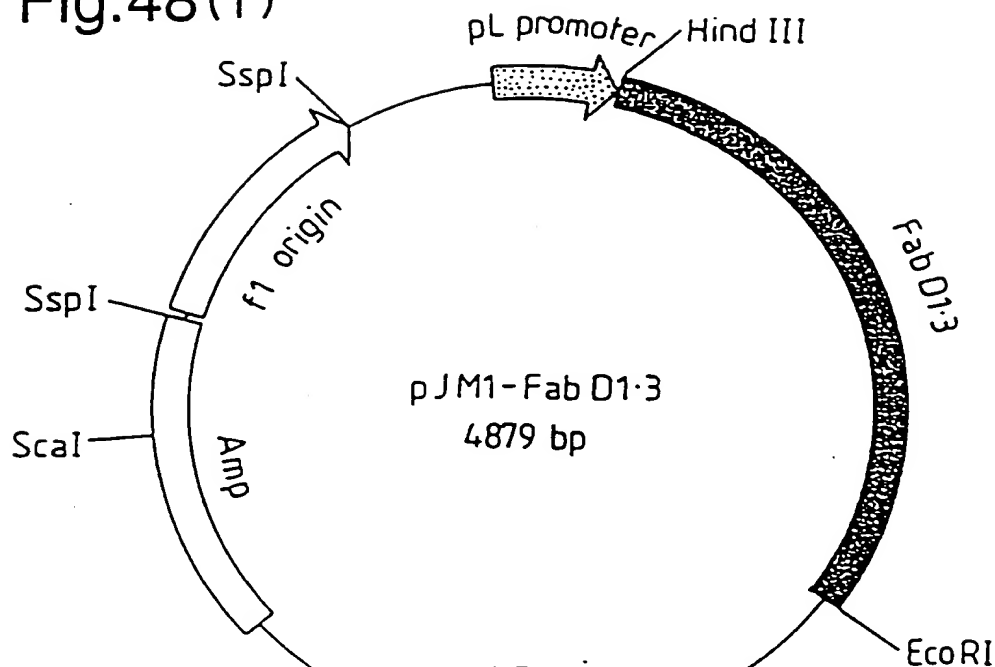


Fig.48(ii)

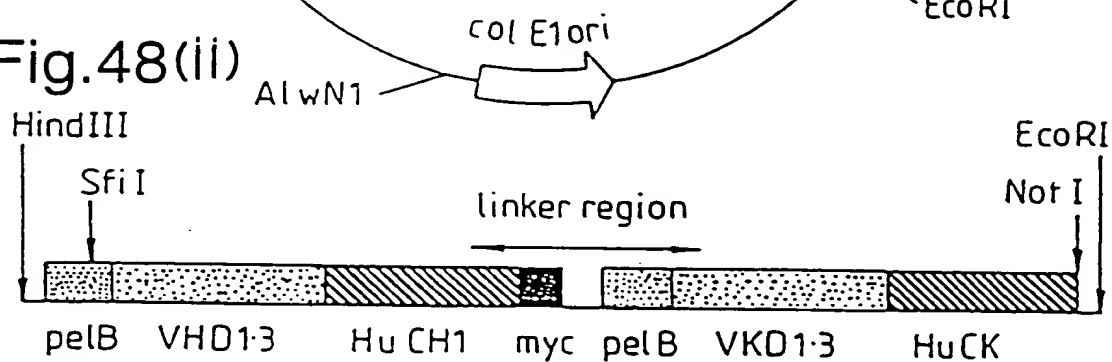


Fig.48(iii)

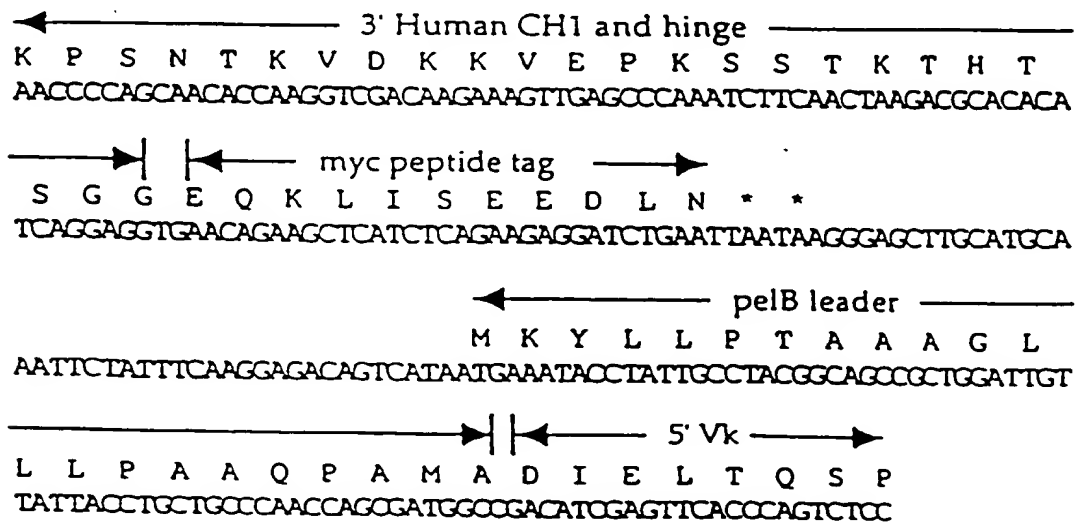
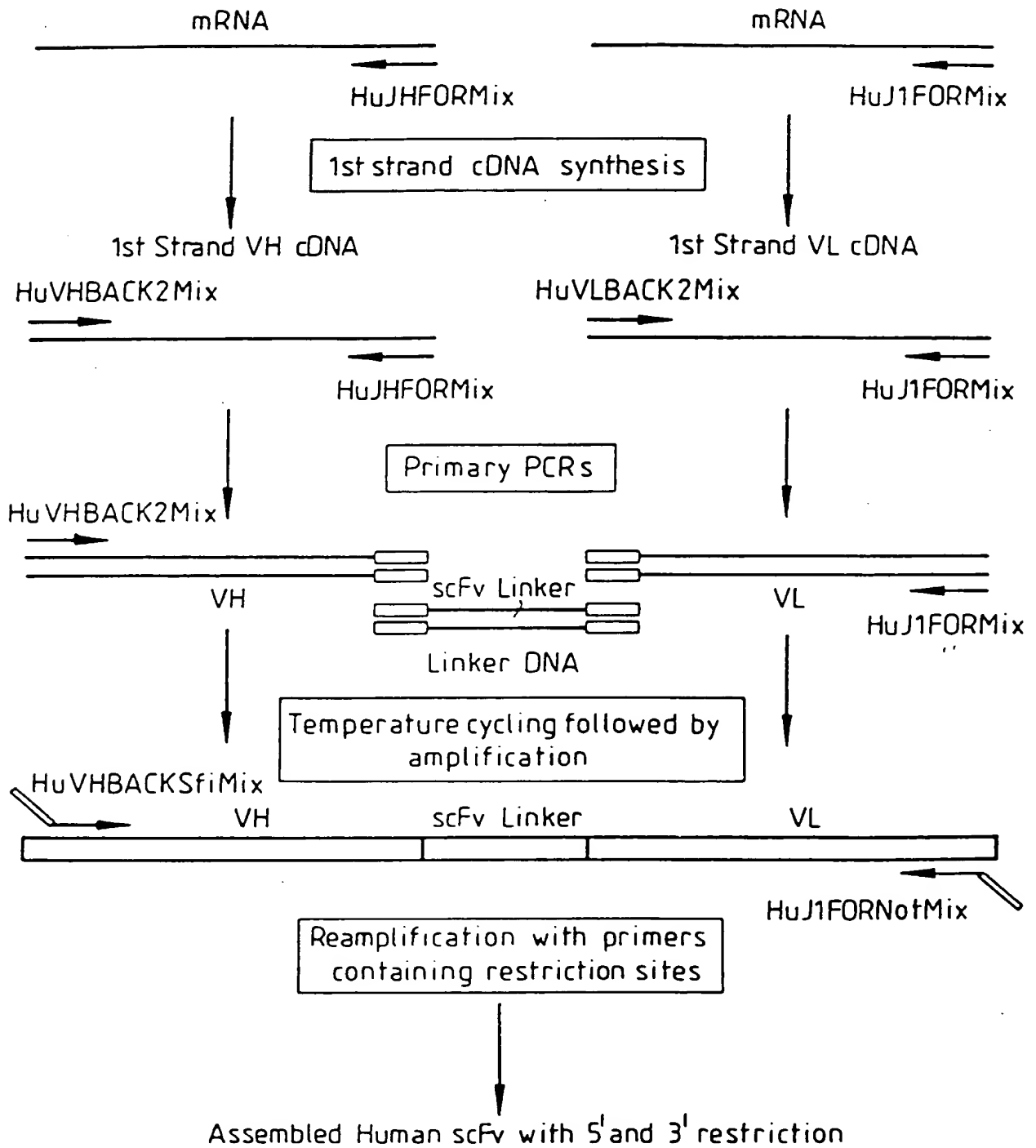
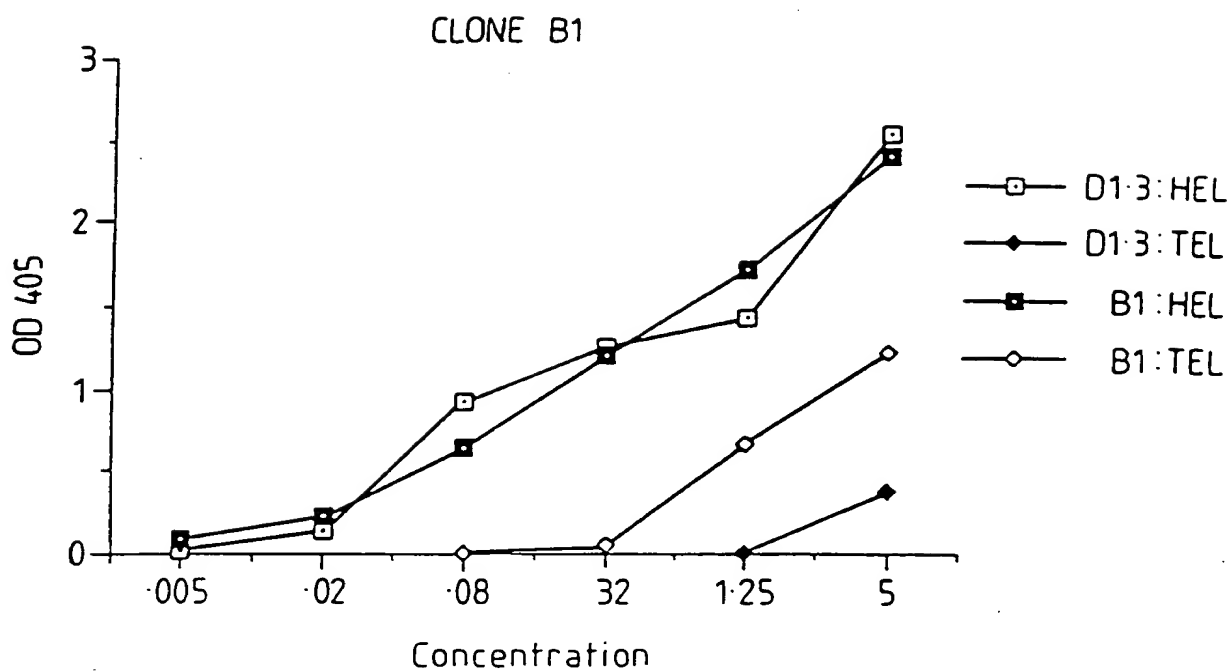


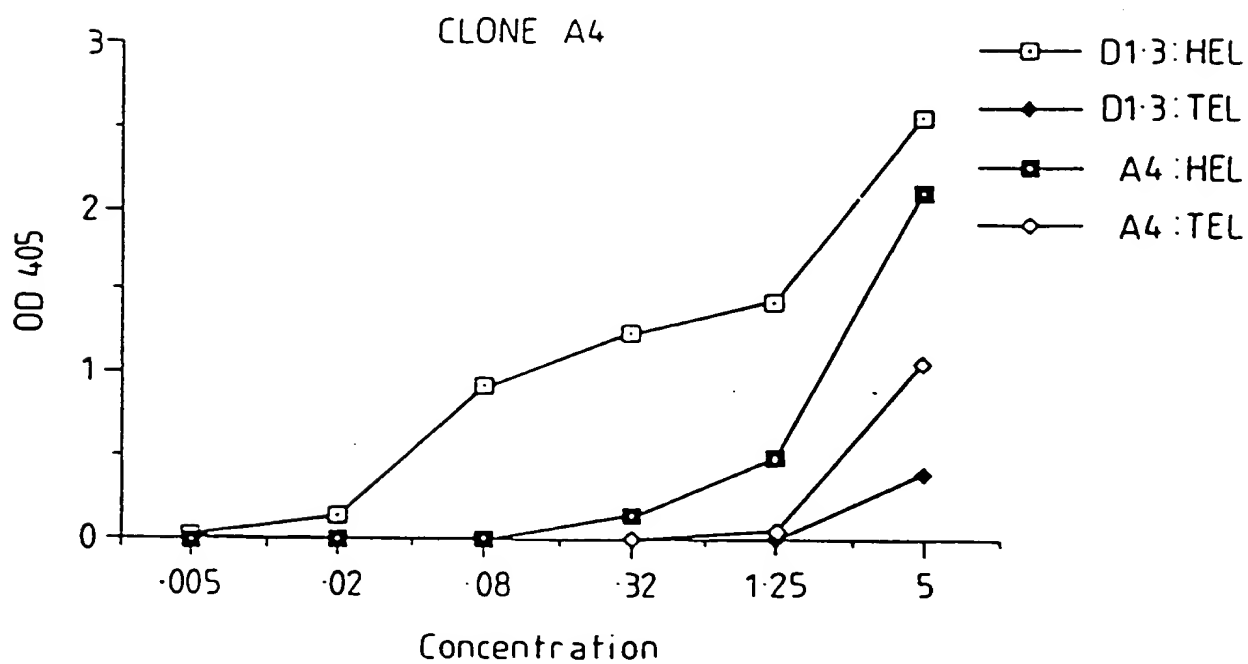
Fig.49.



# Fig.50(i)



# Fig.50(ii)



554749-10139  
554749-10139

Fig.51.

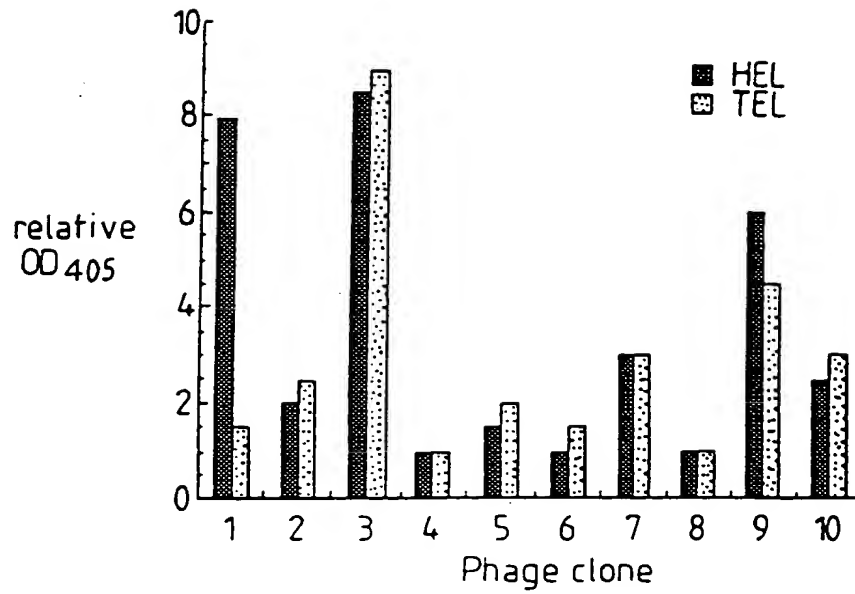


Fig.53.

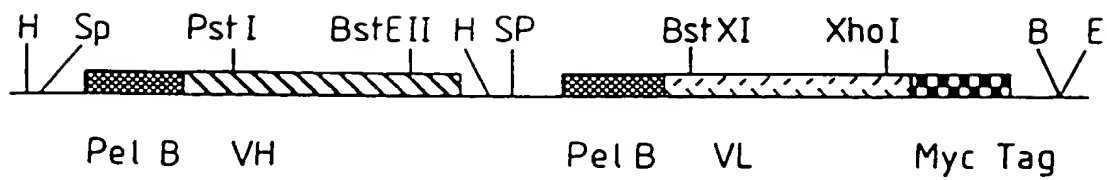


Fig.52.

CDR 1

CDR 2

D1.3 DIQMTQSPASLSASVGETVTITCRASGNIHNYLA WYQQKQKSPQLLVYYTTTLAD  
M1F DIELTQSPSSLSASLGERVSLTCRASQDIGSSLN WLQQEPDGTIKRLIYATSSLDLS  
M21 DIELTQSPALMAASPGEKVTITCSVSSSISSSNLHWYQQSETSPKPWIYGTSNLS

CDR 3

D1.3 GVPSRFSGSGGTQYSLKINSLQPEDFGSYQCQHFWSPTPTFGGKLEIKR  
M1F GVPKRFSGRSGSDYSLTISSLESEDFVDYYCLQYAGSPWTFGGGKLELKR  
M21 GVPVRFSGSGGTSYSLTISSMEAEADAATYYCQWSSYPPLTFGAGTKLEIKR